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THE COURSE OF STONE CELL FORMATION IN PEAR FRUITS

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(WITH EIGHT FIGURES)

Introduction

CRIST and BATJER'S (7) analyses of the isolated grit cell clusters of pear fruits show these structures to be approximately three-fourths lignocellulose, of which about a third is lignin. This quantitative relationship was consistent enough in the several samples tested to justify quantitative estimates of the grit cell content of the fruits by lignocellulose determinations. Their histological studies revealed cell wall thickenings 20 days after blossom fall, and chemical analyses indicated lignification occurring two or three days after blossoming.

The accumulation of lignocellulose as a percentage of the dry weight proceeded rapidly for about four weeks, reaching a concentration of half the fruit's dry weight, then began sharply to decrease until at harvest time it amounted to about a fifth of the dry weight. During the period of lignification there occurred a steady decline in the relative amounts of alcohol extractable material, which reached a minimum at the time the lignocellulose reached a maximum. At this point, indicated by a decrease in the percentage of lignified tissue, began the accumulation of alcohol extractable material which continued for the rest of the growing season.

How perfectly the relative amount of alcohol extractable substances can be a reciprocal of the amounts of lignocellulose, in the two varieties of pears studied, appears in figure 21 of CRIST and BATJER'S report. For example, the Kieffer fruits on May 18 had an alcohol extractable content of 55 per cent. of the dry weight, which dropped to nearly 25 per cent. in the latter part of June, and then increased steadily, reaching a concentration of 55 per cent. again by September 2. The alcohol extract is composed largely of sugars. The lignocellulose started with a concentration of about 25 per

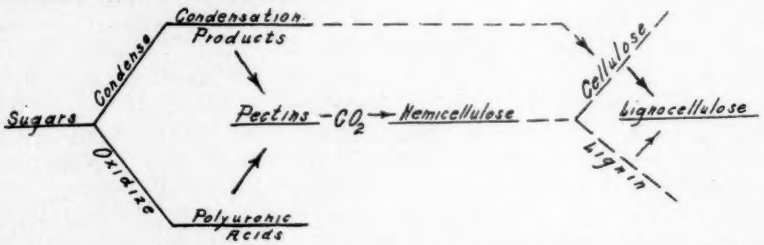
cent., reached a maximum the last of June of over 55 per cent. and then dropped to 25 per cent. by September.

This same interrelation of these materials was found in the Bartlett pear, which in comparison with the Kieffer is less "gritty" and shows a lower percentage of lignocellulose, coupled with a higher percentage of alcohol extractable substances.

It is also interesting that the "conversion point," so to speak, in this variety is about a week earlier, at which time the relative amounts of these materials correspond closely with those of the Kieffer. The regularity of this typical change in relative concentrations for all varieties of pear fruits studied, grown under different cultural conditions, in widely separated localities, for several unlike seasons, establishes it as a fundamental basic phenomenon and as the inherent order of these changes in growing pear fruits.

In general, the chemical changes incident to cell wall formation are in the direction of lignification. So far as the author is aware, the literature presents no clear cut evidence of a reversal in this order. The suggestion of CRIST and BATJER, however, that such a reversion occurs incident to the development of grit cells in the pear, lignocellulose being transformed to sugars, makes desirable a further study of the chemical changes occurring in these structures.

The results of studies of several investigators suggest a theoretical course of events in the process of lignification of plant tissues which may be indicated by the following diagram:



It is known that the first products of photosynthesis, translocated as monosaccharides or disaccharides, are sugars (**9, 21, 29**) and that the end products in the lignification of plant tissues are lignocelluloses (**11, 16, 17**). The course the sugars follow and the changes they undergo to reach this final stage is a point of fundamental concern. Many investigators have sought, chiefly on woody material, a clue to this process (**3, 5, 8, 10, 11, 21**). That the sugars condense to form polysaccharides, there is no doubt (**25, 27**). That they oxidize to form sugar acids known as polyuronic acids is supported by the results of certain research (**25, 27**).

The polyuronic acids, galacturonic, glucuronic, and others, are of particular interest because they combine with certain condensation products (arabinose, galactose) (**12, 13, 15**) to form pectins, and also, they seem to be a part of the hemicelluloses (**5, 24**).

Change of pectin to hemicellulose was fairly well established by CANDLIN and SCHRYVER (**5**). In their investigations on chemical changes taking place in cell wall substances during lignification, they group the substances accompanying cellulose in cell walls into three classes: pectins, hemicelluloses, and lignins. They were able to decarboxylate pectins with the formation of hemicelluloses which resembled in all respects the hemicelluloses isolated directly from timbers. Their results indicate that decarboxylation takes place when plant tissues lignify. They were unable, however, to establish a direct connection between pectins and lignins.

The aim of this investigation was to seek additional evidence of movement to right as indicated by the foregoing diagram (*i.e.*, from sugars through pectins, etc., to lignocellulose), to examine the possibility of a reversal of the direction of movement, and also to further the objective of relieving pear fruits generally, those of the Kieffer in particular, from the burden of grittiness in quality.

Technical methods

Sampling.—The material used for lignocellulose determinations was sampled as described by CRIST and BATJER (**7**); that is, transverse segments were cut from the center of each fruit, the loculi of the carpels removed, and the segments dried in an electric oven at 65° C. For carbohydrate and pectin analyses similar portions of the fruits were taken. These were finely ground in a meat grinder and thoroughly mixed. Small amounts were placed in weighing bottles for dry weight determinations, made at 95° C. Samples consisting of 25 grams of this material were quickly weighed and dropped into mason jars containing boiling 95 per cent. alcohol of sufficient volume to give a final concentration, including the moisture of the sample, of 80 per cent. alcohol. Boiling was continued for 10 minutes, after which the jars were sealed and stored pending analysis. Reductions obtained in similar samples boiled 10, 30, and 60 minutes indicate that complete extraction of the reducing substances was obtained by boiling 10 minutes.

Small amounts of calcium carbonate were added to the first samples to neutralize the acids, but because of the small amounts of acids present and the short period of heating, it seemed that hydrolysis would be negligible. ARCHBOLD (**2**) in a report on work with apples states: "No difference was found in the estimated amounts of sugar in untreated solutions compared with solutions treated with calcium carbonate during the hot extraction or with ammonia during both cold and hot extractions. Hydrolysis during

alcohol extraction is therefore presumed to be negligible." The first season's study showed that the calcium carbonate interfered with the pectin determinations by neutralizing the weak acid used in extracting total pectins. Therefore it was omitted in subsequent samples.

Analysis.—Benzene extractions, alcohol extractions, water extractions, and alkali extractions were made and the cellulose and lignin determinations secured as described by CRIST and BATJER.

Total sugars, sucrose, dextrins, starch, and hemicellulose were determined as outlined by the committee on chemical methods of the American Society of Plant Physiologists (**31**).

Pectins as calcium pectate were determined by the method established by CARRÉ and HAYNES (**6**) and employed by APPLEMAN and CONRAD (**1**).

During the growing seasons of 1930 and 1931, reducing substances were determined by the modified SHAFFER-HARTMANN titration method (**30, 33**). The sugar solutions obtained by taking up the alcohol extracts in water, being quite free of coloring matter, were used directly to avoid loss of sugars which might be thrown down in the clearing process (**4**).

Investigations by PHILLIPS (**28**) indicate that with certain materials the SHAFFER-HARTMANN method gives high values. He cites SULLIVAN (**34**) as finding that iodine liberated in the presence of plant extracts may be absorbed by some constituent of the extract, such as phlorizin, causing an error in the determination of the reducing copper. To test this possibility, reductions were determined on the water solutions of the alcohol extracts of the 1933 samples after being reduced at 80° C. for 30 minutes, first by the modified SHAFFER-HARTMANN titration method in which titration was carried out in the presence of the plant extract; and second, by the volumetric thiosulphate method (**17**) in which the cuprous oxide is separated by means of an asbestos mat in a Gooch crucible and titrated free of the plant extracts. To check further on this point, the filtrate from which the cuprous oxide had been separated was titrated at once by the modified SHAFFER-HARTMANN method, with the results presented in tables I and II.

These data show clearly that with these extracts the modified SHAFFER-HARTMANN method gives values greater than the amounts of copper reduced warrant. Indications are that this difference is due to some substance in the plant extract which does not reduce the copper, but probably behaves as suggested by SULLIVAN (**34**). This substance, whatever its nature, seems to be present in larger amounts in extracts from the earlier samples, which are more highly colored and contain larger proportions of skin to flesh of the fruit.

This test was repeated on cleared solutions taken from apple tissue. Apparently some of the reducing substances were removed by clearing as the amounts of reduced copper were slightly lower. The greatest loss was in

TABLE I

AVERAGE CC. OF 0.1 N SODIUM THIOSULPHATE SOLUTION REQUIRED TO TITRATE REDUCTION OF FEHLING'S SOLUTION SECURED WITH 50 CC. OF THE ALCOHOL SOLUBLE EXTRACT OF APPLE FRUITS

DATE OF SAMPLING*	UNCLEARED SOLUTIONS							CLEARED SOLUTIONS WITH 0.5 GRAMS OF NEUTRAL LEAD ACETATE PER 100 CC.			
	FIRST TEST			SECOND TEST							
	TITRATION IN PRESENCE OF PLANT EXTRACT BY SHAFFER-HARTMANN METHOD	REDUCED COPPER SEPARATED FROM PLANT EXTRACT AND TITRATED BY VOLUMETRIC THIOSULPHATE METHOD	DIFFERENCE BETWEEN THE TWO METHODS	TITRATION IN PRESENCE OF PLANT EXTRACT BY SHAFFER-HARTMANN METHOD	REDUCED COPPER SEPARATED FROM PLANT EXTRACT AND TITRATED BY VOLUMETRIC THIOSULPHATE METHOD	SHAFFER-HARTMANN TITRATION OF FILTRATE FROM VOLUMETRIC THIOSULPHATE METHOD	COPPER TITRATION PLUS FILTRATE TITRATION	TITRATION IN PRESENCE OF PLANT EXTRACT BY SHAFFER-HARTMANN METHOD	REDUCED COPPER SEPARATED FROM PLANT EXTRACT AND TITRATED BY VOLUMETRIC THIOSULPHATE METHOD	SHAFFER-HARTMANN TITRATION OF FILTRATE FROM VOLUMETRIC THIOSULPHATE METHOD	COPPER TITRATION PLUS FILTRATE TITRATION
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
May 27				3.4	1.0	2.4	3.4	1.9	0.3	1.5	1.8
June 4	4.7	2.4	2.3	4.8	2.4	2.4	4.8	3.2	2.0	1.0	3.0
10	4.5	3.0	1.5	4.8	3.0	1.7	4.7	3.5	2.6	0.7	3.5
16	6.9	5.7	1.2	6.9	5.3	1.6	6.9	5.7	5.1	0.8	5.9
24	7.9	6.7	1.2	8.0	6.8	1.0	7.0	6.0	6.2	0.4	6.6
30	8.2	7.7	0.5	8.3	7.7	0.7	8.4	7.6	7.4	0.5	7.9
July 7	8.4	8.1	0.3	8.7	8.3	0.6	8.9	8.4	8.0	0.4	8.4
14	9.5	9.5	.....	9.9	9.4	0.5	9.9	9.6	9.2	0.4	9.6
25	10.7	9.7	1.0	11.2	10.7	0.6	11.3	11.5	11.3	0.2	11.5
Aug. 9	11.8	11.8	.....	11.7	11.4	0.4	11.8	11.0	10.7	0.4	11.1
Sept. 9	14.5	14.3	0.2	14.6	14.0	0.4	14.4	13.9	13.6	0.2	13.8
Oct. 9	13.3	13.5	0.3	13.8	13.4	0.3	13.7	13.4	13.1	0.2	13.3

* 80 grams fresh weight for each sample.

the SHAFFER-HARTMANN titrations and shows that about half of this unknown material was taken from the solutions by clearing. It would seem from these facts that this substance is associated with the skin or pigments in both apple and pear fruits.

Reducing substances in the 1933 samples were determined by the volumetric thiosulphate method, as described (**17**), except that reduction was carried out at 80° C. for 30 minutes by means of a hot water bath.

TABLE II

AVERAGE CC. OF 0.1 N SODIUM THIOSULPHATE SOLUTION REQUIRED TO TITRATE REDUCTIONS OF FEHLING'S SOLUTION SECURED WITH 50 CC. OF THE ALCOHOL SOLUBLE EXTRACT OF PEAR FRUITS

DATE OF SAMPLING*	TITRATION IN PRESENCE OF PLANT EXTRACT BY SHAFFER-HARTMANN METHOD	REDUCED COPPER SEPARATED FROM PLANT EXTRACT AND TITRATED BY VOLUMETRIC THIOSULPHATE METHOD	FILTRATE FROM VOLUMETRIC THIOSULPHATE METHOD TITRATED BY SHAFFER-HARTMANN METHOD	COPPER TITRATION PLUS FILTRATION TITRATION
	cc.	*cc.*	*cc.*	*cc.*
May 20	1.6	0.3	0.6	0.9
27	3.2	1.5	1.5	3.0
June 4	3.7	2.2	0.9	3.1
10	3.1	2.3	0.9	3.2
16	4.1	2.9	0.6	3.5
24	3.8	3.0	0.4	3.4
30	3.7	3.0	0.2	3.2
July 7	5.2	4.6	0.2	4.8
14	6.3	5.4	0.3	5.7
25	7.3	6.3	0.3	6.6
August 9	9.6	8.7	0.1	8.8
September 9	15.8	14.7	0.1	14.8
October 9	16.7	16.3	.....	16.3
Novemebr 4	19.0	18.6	0.2	18.8

* 80 grams fresh weight per sample.

Hardness of fruits.—Hardness of the fruits was obtained on each sampling date by means of a pressure tester (**20**). A plunger of 4/16 inch diameter was substituted for the regulation 5/16 inch one, to permit readings on the early hard fruits. The pressures thus obtained may be converted, approximately, over to 5/16 plunger values, by the factors 1.42 when skin is removed and 1.35 when skin is not removed. These factors were derived by determining the pressure for each plunger on the same fruits. As the ratio varies a little, being greater with the softer fruits, these factors are only approximate, but serve to give an estimate of the hardness of the early fruits.

Procedure and results

During the growing season of 1930, samples for chemical analysis were taken periodically of Kieffer and Bartlett pears and Wagener apples from vigorous, productive trees.

Changes in the amount of lignocellulose, total sugars, reducing sugars, total pectins and soluble pectins, as percentages of the dry weight were

determined. Figure 1 compares graphically the changes in lignocellulose and total sugars in the three fruits studied.

A more detailed story was desired of the changes occurring in the Kieffer fruits. Consequently in 1931 fruits of Kieffer pear were sampled every third day from June 2 until July 17 and less frequently thereafter, as long as any fruits remained on the tree. As many as 1200 fruits were required to furnish enough material for a single sample on the earlier dates and a minimum of 25 fruits was used in each sample.

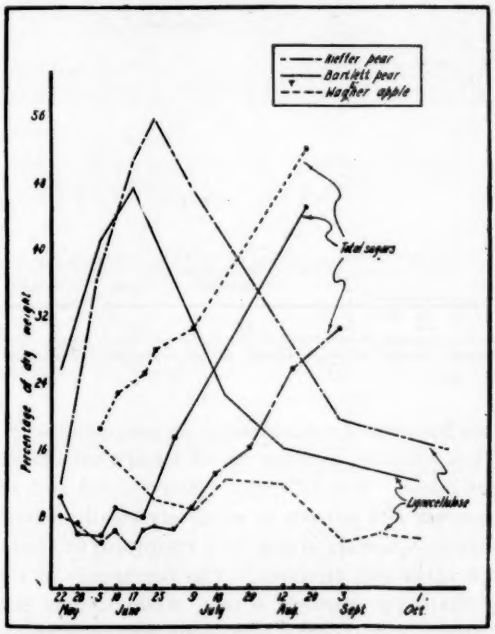


FIG. 1. Seasonal course of total sugars and lignocellulose as percentage of dry weight in fruits of different "grittiness," 1930.

Quantitative determinations of the constituents of the Kieffer pear fruits secured during the growing season of 1931 are presented in table III and graphically in figure 2. The changes in lignocellulose, sugars, and pectins are similar to those secured in 1930.

Changes in relative amounts of lignocellulose in the Kieffer (fig. 1 and 2), consisting of a very rapid accumulation during the first four weeks after fruit setting followed by a less rapid decrease, are identical with those found by CRIST and BATJER (7). The accumulation of lignocellulose is accompanied by a decrease in the amounts of total and reducing sugars, until

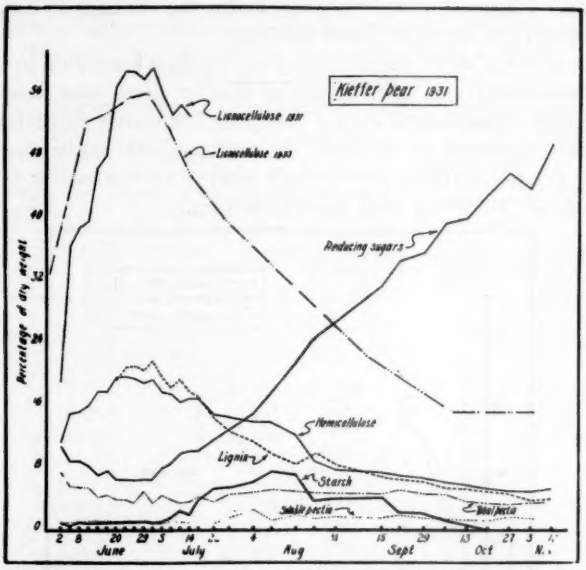


FIG. 2. Seasonal course as percentage of dry weight of several constituents of Kieffer pear fruit, 1931.

a few days before the peak of concentration of lignocellulose is reached. The sugar curves then indicate a piling up of sugars coincident with the decrease in lignocellulose. The difference between total and reducing sugars is reported as sucrose and persists in relatively small amounts. The curve for sugar concentrations could almost be a reciprocal of the curve for lignocellulose both in value and direction. The percentage of total pectins decreases during the early growing season, while soluble pectins increase. After the first part of July, both show a slight gradual increase. The difference between total and soluble pectins is reported as protopectin (**6**), which goes over to soluble pectins during the life of the pear.

COMPARISON OF CHANGES IN THE APPLE AND PEAR FRUITS

That "grit cells" are composed chiefly of lignocellulose, and that their formation is a result of lignification which may be measured quantitatively by lignocellulose determinations has been established by CRIST and BATJER.

As would be expected from their findings, the percentage of lignified tissue does not increase in the "grit cell"-free Wagener apple fruits (fig. 1). Although lignocellulose starts at a concentration equal to that in the pear, it decreases continuously throughout the season, except for a short

TABLE III

PERCENTAGE COMPOSITION OF KIEFFER PEAR FRUITS, 1931, DRY WEIGHT BASIS

DATE OF SAMPLING	MOISTURE	DRY WT. OF FRUIT	DRY WT. OF ALC. EXT.	REDUCING SUGARS	DEXTRINS	STARCH	HEMICELLULOSE	RESIDUE*	LIGNIN	PECTIN	TOTAL PECTIN
	%	%	%	%	%	%	%	%	%	%	%
June 2	84.87	15.13	51.67	10.63	0.84	0.76	11.14	19.07		0.75	7.30
5	84.27	15.73	42.82	8.46	0.61	0.54	14.91	36.20		0.99	5.61
8	83.54	16.46	43.25	8.62	0.54	0.77	15.01	39.10		0.75	5.52
11	83.43	17.57	42.19	8.12	0.64	0.67	15.93	39.79		0.87	5.42
14	81.23	18.77	34.62	6.92	0.62	0.74	17.82	46.22		0.92	4.08
17	78.92	21.08	34.83	7.44	0.80	0.66	17.64	48.15		0.74	4.21
20	78.26	21.74	32.58	6.20	0.42	0.63	19.59	41.74	19.20	1.07	3.95
23	77.63	22.37	29.96	6.25	0.83	0.75	19.63	40.22	20.70	1.10	3.85
26	77.58	22.42	30.50	6.19	0.71	0.66	19.51	41.62	20.80	1.10	3.68
29	77.53	22.47	31.64	6.36	1.00	0.85	18.82	42.87	20.30	0.85	4.75
July 2	76.83	23.17	29.77	6.38	1.07	0.75	19.23	42.90	21.28	0.71	3.68
5	75.85	24.15	33.28	7.94	1.80	0.87	17.75	42.98	19.80	0.71	4.30
8	76.34	23.66	34.79	8.45	.95	1.62	17.50	40.01	18.36	1.19	3.89
11	76.55	23.45	36.67	9.56	1.07	2.02	16.30	38.68	19.24	1.05	3.58
14	77.96	22.04	37.60	10.08	1.44	1.72	16.79	37.96	17.86	1.03	3.64
17	78.60	21.40	37.80	10.00	1.16	3.85	16.98	35.80	17.36	1.00	4.11
22	76.54	23.46	39.53	11.30	1.14	5.19	14.78	32.74	14.45	0.65	3.79
27	77.00	23.00	44.69	12.60	1.60	5.40	14.60	28.97	12.40	1.61	4.34
August 4	78.00	22.00	49.42	14.72	1.75	6.10	13.97	26.66	11.05	2.68	4.62
10	79.57	20.43	54.76	17.79	2.05	7.61	13.77	24.20	9.81	1.27	5.08
18	79.77	20.23	59.86	21.38	1.76	7.06	12.12	21.14	8.36	1.97	5.03
24	80.00	20.00	63.53	24.55	1.26	3.82	8.88	19.14	9.82	1.40	4.61
31	80.36	19.64	64.73	26.73	1.80	4.00	7.80	17.53	8.53	1.80	4.73
September 15	80.91	19.09	71.83	31.40	1.32	4.12	7.65	14.27	6.86	1.61	4.69
21	82.59	17.41	71.90	34.73	1.44	2.11	7.08	14.18	6.54	1.94	5.15
29	82.58	17.42	76.23	35.87	0.99	2.10	6.53	11.97	6.05	1.60	4.58
October 6	82.65	17.35	80.23	39.59	0.93	1.14	5.94	10.79	5.44	1.47	4.41
13	82.18	17.82	80.61	40.17	0.69	0.39	5.79	10.65	5.27	1.42	3.73
20	82.26	17.74	84.72	43.29	0.66	trace	5.38	9.72	4.67	1.26	3.67
27	82.16	17.84	86.49	46.07	0.61	trace	5.03	9.37	4.34	1.76	3.91
November 3	82.62	17.38	87.27	44.76	0.87	0.13	4.85	9.42	3.96	1.62	3.85
10	84.00	17.00	98.85	49.87	0.62	0.25	5.37	10.13	4.22	1.35	3.88

* Fraction of sample remaining after carbohydrate extraction.

period in July. Accumulation of sugars is not delayed as in the pear fruits, but proceeds at a uniform rate from the very start.

Changes in hemicelluloses in the Kieffer pear (fig. 2) follow closely changes in lignin. Although the changes are of a different character in the apple, this relation of hemicellulose to lignin seems to hold, as WIDDOWSON (**37**) also shows a rapid decrease in the percentage of hemicellulose in the early life of Bramley's seedling apple, followed by a less rapid decline during the remainder of the growing season. Changes in starch concentration found in the apple by WIDDOWSON (**37**) and TETLEY (**35**) have the same character as those found in the Kieffer pear.

Changes in the Bartlett pear are similar to those in the Kieffer, but with a general shortening of the whole process. Lignocellulose curves start at a higher concentration, reach a minimum earlier in the season and fall to a lower level than those for the Kieffer (fig. 1). Although total sugars show a decrease for the first two weeks after fruit set, they are less pronounced than in the Kieffer and accumulation of sugars starts about 10 days earlier (fig. 1). The greater amount of total pectins in the Bartlett seems to be due to a greater quantity of protopectin. The character of the pectin changes is much like that found in Kieffer.

Inspection of figures 1 and 2 reveals a critical "point of change" in the pear fruits where those constituents which have been accumulating decrease suddenly, and those materials which have been decreasing, begin to accumulate. This "conversion point" occurs about ten days earlier in the Bartlett fruit than in the Kieffer.

In the Wagener apple there is a rapid accumulation of sugars, showing a steady increase in sucrose, and a gradual decrease in lignocellulose from the very earliest sampling. As in the pear fruits, protopectin goes over to soluble pectin early in the season. The increase in both total and soluble pectins during the last part of the growing season distinguishes pectin changes in the apple qualitatively from those in the pear.

ONSLOW (**27**) and CRIST and BATJER show lignin to account consistently for about one-third of the lignocellulose. These data coincide with their findings and, as would be expected, lignin changes are qualitatively the same as lignocellulose (fig. 2).

Hemicellulose changes, presented graphically in figure 2, are almost identical with lignin changes both in amounts and direction, except for the period of starch concentration from July 17 to August 24. During this time hemicelluloses do not decrease as rapidly as does the lignin and they maintain a difference of about 4 per cent. of the dry weight. It may be significant that this over-rapid decrease in lignin, and slowing of the hemicellulose decline, coincide closely with the high concentration of starch.

TABLE IV

PERCENTAGE COMPOSITION OF KIEFFER PEAR FRUITS IN STORAGE AT 33° F., DRY WEIGHT BASIS

	MOISTURE	DRY WT. OF FRUIT	DRY WT. OF ALC. EXT.	REDUCING SUGARS	DEXTRIN	STARCH	HEMICELLULOSES	RESIDUE*	LIGNIN	PECTIN	TOTAL PECTIN
Harvested and placed in storage September 29, 1931											
	%	%	%	%	%	%	%	%	%	%	%
Sept. 29	82.30	17.70		37.92	0.99	0.67	6.39	14.07	6.67	2.32	4.76
Oct. 13	82.42	17.58		38.81	0.86	0.31	6.34	14.99	6.84	2.50	4.64
Nov. 26	82.70	17.30		38.84	0.73	0.45	6.76	15.77	7.29	1.79	5.82
Dec. 17	83.31	16.69		39.98	0.89	0.34	6.04	16.19	7.73	2.07	5.05
Jan. 7	83.13	16.87		40.02	1.30	0.35	6.82	15.52	7.51	1.98	5.88
" 21	84.37	15.63		41.59	1.36	0.31	6.50	16.89	7.72	2.73	6.77
Feb. 11	84.90	15.10		43.97	1.08	0.42	6.42	16.85	7.53	3.19	5.56
" 25	84.30	15.70		42.47	1.24	0.98	5.57	15.75	7.16	3.48	5.32
Harvested and placed in storage October 13, 1931											
Oct. 13	82.44	17.56	79.72	42.14	0.79	0.22	5.55	12.41	5.33	2.22	4.55
Nov. 26	82.54	17.46	83.61	44.26	0.75	0.28	5.77	12.91	4.93	1.65	4.17
Dec. 17	83.00	17.00	85.29	44.70	0.78	0.24	5.84	12.91	6.25	1.81	4.70
Jan. 7	83.10	16.90	85.02	45.20	.....	0.32	5.39	13.64	6.44	1.46	4.73
" 21	84.21	15.79	85.54	45.22	1.16	0.39	6.48	15.52	7.16	2.00	.....
Feb. 11	83.60	16.40	83.92	45.85	1.16	0.87	5.60	14.31	7.12	2.53	5.70
Feb. 25	83.57	16.43	84.50	45.74	0.90	0.35	5.49	13.64	6.09	2.73	4.54
Harvested and placed in storage October 27, 1931											
Oct. 27	82.40	17.60		45.79	0.68	0.18	4.90	11.87	5.13	1.30	3.88
Nov. 26	82.74	17.26		46.16	0.63	0.28	5.04	11.30	5.36	1.01	4.00
Dec. 17	83.44	16.56		48.26	0.62	0.28	5.89	12.43	5.51	1.87	4.00
Jan. 7	83.72	16.28		48.69	1.11	0.46	5.40	12.20	5.77	1.62	5.52
" 21	84.16	15.84		50.75	0.90	0.22	4.74	12.11	5.19	2.27	5.15
Feb. 11	84.22	15.78		49.10	0.80	0.68	4.08	12.53	5.47	2.10	5.06
" 25	83.78	16.22		47.59	0.81	0.63	4.53	12.75	5.48	2.51	4.26

* Fraction of sample remaining after carbohydrate extraction.

Starch remains insignificant (less than 1 per cent.) until early July when it begins to accumulate rapidly, reaching a concentration of 5 per cent. This high concentration is maintained until the middle of August, after which a uniform decline occurs and starch again becomes insignificant about the middle of October.

Dextrins and soluble starches do not become important at any time. They do, however, follow the general trend of the starches, with concentrations varying from 0.5 to 1.5 per cent. of the dry weight.

Carbohydrate residue curves are similar to lignocellulose curves. Analyses show this residue to be composed almost entirely of lignocellulose.

RIVIÈRE and BAILHACHE (**32**) report that ripening, as measured by the sugar content, is progressive from the stem end to the calyx end in the three varieties of pears studied; namely, Beurré Hardy, Angouleme, and Comice. If this is true in the Kieffer pear, sampling which includes the whole fruit would be more representative of the sugar content than that taking only the mid-section. For this reason, and to permit the expression of the various constituents in absolute quantities per fruit, the samples taken in 1933 of Kieffer pear fruits and Wagener apple fruits from the college orchard comprised whole fruits from which the loculi of the carpels with their contents were removed. The average weights and volumes of the fruits were determined at each sampling.

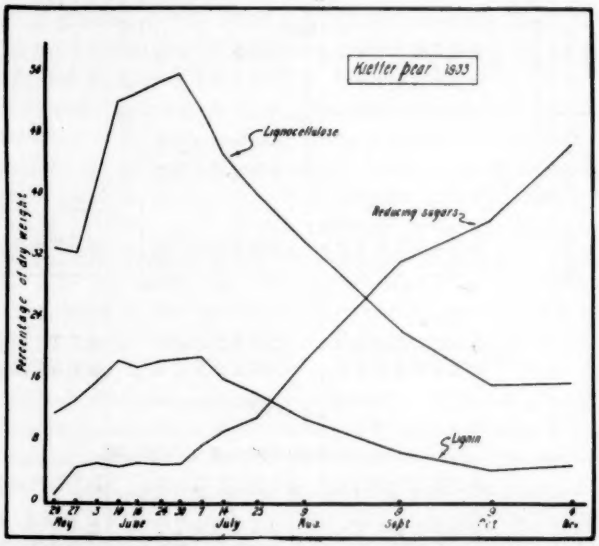


FIG. 3. Seasonal course of reducing sugars, lignocellulose, and lignin as percentage of dry weight. Kieffer pear fruit, 1933.

The findings secured in 1933 are presented graphically in figures 3 and 4 on a dry weight basis and in figures 5 and 6 as absolute amounts of

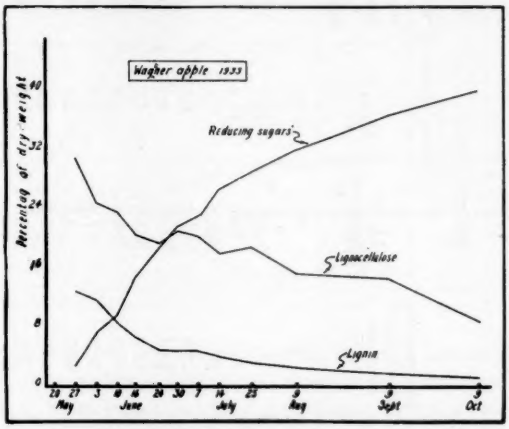


FIG. 4. Seasonal course of reducing sugars, lignocellulose, and lignin as percentage of dry weight. Wagener apple fruit, 1933.

the constituents per fruit. On a dry weight basis the findings are in accord with those of 1930 and 1931. On an absolute amount per fruit basis, an entirely different picture of the changes in the constituents is obtained.

Inspection of figures 3 and 5 reveals the deceptiveness of expressions on the basis of percentage of dry weight. None of the constituents of the

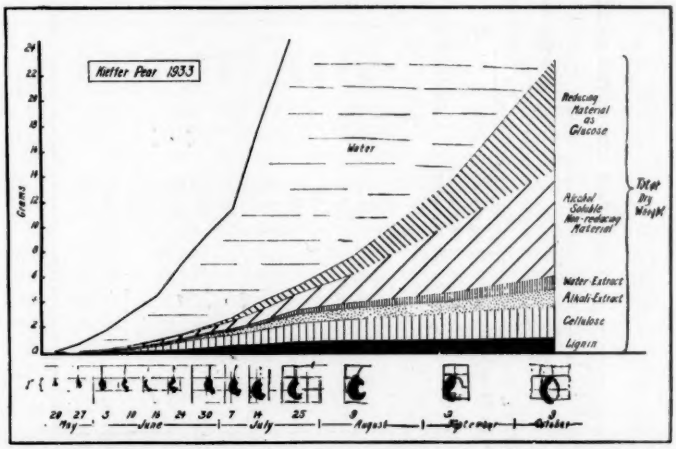


FIG. 5. Seasonal course of actual weight of constituents. Kieffer pear fruit, 1933.

TABLE V

PERCENTAGE COMPOSITION OF KIEFFER PEAR AND WAGENER APPLE, 1933, DRY WEIGHT BASIS

DATE OF SAMPLING	KIEFFER PEAR				WAGENER APPLE			
	DRY WEIGHT PERCENTAGE OF FRESH WEIGHT	PERCENTAGE OF DRY WEIGHT			DRY WEIGHT PERCENTAGE OF FRESH WEIGHT	PERCENTAGE OF DRY WEIGHT		
		LIGNO-CELLULOSE	LIGNIN	REDUCING MATERIAL AS DEXTROSE		LIGNO-CELLULOSE	LIGNIN	REDUCING MATERIAL AS DEXTROSE
	%	%	%	%	%	%	%	%
May 20	11.1	33.8	11.7	1.0	.....	.....	.....	.....
" 27	12.2	32.6	13.4	4.5	13.5	30.5	12.5	2.8
June 4	15.9	42.3	15.8	5.2	12.7	24.3	11.8	7.2
" 10	19.2	52.2	18.7	4.7	12.7	23.3	8.2	9.1
" 16	21.3	53.5	17.8	5.3	13.9	20.2	6.5	14.8
" 24	22.0	55.6	18.4	5.3	14.4	19.2	4.9	18.4
" 30	22.0	56.0	18.9	5.3	13.9	20.6	4.7	21.7
July 7	23.4	51.5	17.4	7.9	14.2	20.0	4.8	23.1
" 14	22.0	45.5	16.2	9.6	14.0	17.9	4.0	26.3
" 25	20.0	40.2	14.2	11.2	14.5	18.8	3.3	29.0
August 9	18.7	34.3	11.0	18.2	14.2	15.2	2.4	31.7
September 9	18.5	22.3	6.9	31.3	15.1	14.5	1.9	36.6
October 9	17.4	15.5	4.3	37.2	13.5	8.9	1.1	39.1

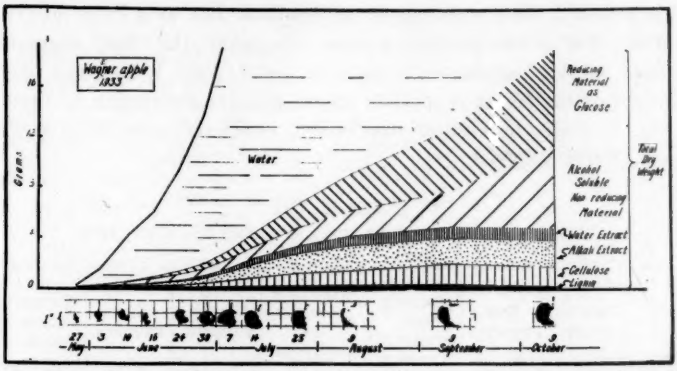


FIG. 6. Seasonal course of actual weight of constituents. Wagener apple fruit, 1933.

pear fruits decreases, but each one actually increases throughout the growing season. Figure 5 shows clearly that the apparent increase and decrease in lignocellulose, when expressed on a dry weight basis, is due only slightly to changes in the rate of accumulation of lignin and cellulose, and principally to changes of the total dry weight. The chief variable of the dry

TABLE VI

CONSTITUENTS OF KIEFFER PEAR IN GRAMS PER FRUIT, 1933

DATE OF SAMPLING		MOISTURE	DRY WEIGHT	ALCOHOL SOLUBLE NON-REDUCING EXTRACT	REDUCING MATERIAL AS DEXTROSE	WATER EXTRACT	ALKALI EXTRACT	LIGNO-CELLULOSE	LIGNIN
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
May	20	0.08	0.01	0.003	0.0001	0.0019	0.0012	0.0011	0.0013
"	27	0.71	0.10	0.040	0.0040	0.0103	0.0121	0.0189	0.0132
June	4	1.52	0.28	0.095	0.0149	0.0166	0.0425	0.0306	0.0905
"	10	2.64	0.62	0.162	0.0293	0.0321	0.0839	0.2101	0.1169
"	16	3.53	0.95	0.232	0.0508	0.0454	0.1202	0.3405	0.1700
"	24	5.13	1.44	0.325	0.0761	0.0645	0.1839	0.5387	0.2662
"	30	7.37	2.07	0.451	0.1093	0.1015	0.2457	0.7721	0.3925
July	7	8.85	2.70	0.608	0.2131	0.1103	0.3438	0.7769	0.6160
"	14	13.83	3.90	0.927	0.3727	0.1724	0.4375	1.1419	0.6336
"	25	20.92	5.23	1.369	0.6465	0.2799	0.6995	1.499	0.8202
August	9	33.34	7.66	2.320	1.3958	0.3596	0.9312	1.7843	0.8474
September	9	60.45	13.71	5.433	4.3308	0.6299	0.9611	2.1299	0.9608
October	9	109.86	23.14	9.773	8.6350	1.0775	1.3871	2.6135	0.9943

weight is the alcohol soluble substances, especially reducing sugars. Lignin and cellulose accumulate faster during May and June than the other dry weight constituents, making a great relative increase of these substances

for this period. They continue to accumulate, but at a little slower rate, during the rest of the growing season. However, the rapid accumulation of alcohol soluble substances beginning about July 1 increases the total dry weight of the fruits so quickly that a relative expression of lignocellulose (fig. 3) indicates, unless cautiously considered, a sudden and rapid decrease of this material.

TABLE VII

CONSTITUENTS OF WAGENER APPLE IN GRAMS PER FRUIT, 1933

DATE OF SAMPLING	MOISTURE	DRY WEIGHT	ALCOHOL SOLUBLE NON-REDUCING EXTRACT	REDUCING MATERIAL AS DEXTROSE	WATER EXTRACT	ALKALI EXTRACT	LIGNO-CELLULOSE	LIGNIN
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
May 27	0.26	0.04	0.0160	0.0011	0.0049	0.0065	0.0128	0.0052
June 4	1.64	0.20	0.0881	0.0145	0.0139	0.0346	0.0489	0.0237
" 10	4.13	0.53	0.2247	0.0483	0.0427	0.1063	0.1245	0.0438
" 16	5.98	0.84	0.2985	0.1263	0.0645	0.2018	0.1728	0.0557
" 24	9.19	1.33	0.3740	0.2465	0.1257	0.3093	0.2560	0.0656
" 30	13.61	1.89	0.4775	0.4135	0.2003	0.4573	0.3917	0.0903
July 7	19.53	2.97	0.6710	0.6871	0.3213	0.7377	0.5973	0.1431
" 14	30.40	4.43	0.9898	1.1687	0.4848	1.1137	0.7940	0.1789
" 25	42.36	6.69	1.5445	1.9462	0.5712	1.6000	1.2585	0.2184
August 9	58.50	9.10	2.1508	2.9048	0.7550	2.0561	1.3859	0.2233
September 9	81.33	13.77	4.7782	5.0919	0.9004	2.0052	2.0147	0.2696
October 9	126.24	18.96	8.6483	7.6017	1.0667	2.2098	1.7275	0.2176

During May and June the increase in size of the fruit is due largely to the formation of new cells and at this time cell wall material accounts for most of the dry weight of the fruit. After the last of June, increase in fruit size is due to expansion of the already formed cells and to enlargement of the intercellular spaces (TETLEY, **36**). This behavior would occasion a progressive decrease in the proportion of cell wall to cell contents, thereby showing a less rapid increase in cell wall materials (lignocellulose) during the remainder of the growing season. Alcohol soluble materials are present chiefly in the vacuoles of the cells and, as would be expected, the larger the cells the greater the proportion of cell inclusions to cell wall constituents. Thereafter, it would seem that the great increase in dry weight is due to cell inclusions and should be considered separately from the cell wall constituents.

Hardness.—Data on hardness of fruits in terms of pounds pressure as determined by the standard pressure tester are presented in figure 8 and table IX.

TABLE VIII

WEIGHT AND VOLUME OF KIEFFER PEAR AND WAGENER APPLE FRUITS, 1933

DATE OF SAMPLING	KIEFFER PEAR			WAGENER APPLE		
	AV. WEIGHT	AV. VOLUME*	SP. GR.	AV. WEIGHT	AV. VOLUME*	SP. GR.
	gm.	*cc.*		*gm.*	*cc.*	
May 20	0.09	0.09	1.00	.....	.....	.....
" 27	0.75	0.73	1.02	0.30	0.30	1.00
June 3	1.92	1.84	1.04	1.84	1.89	.97
" 10	3.51	3.22	1.09	4.66	4.91	.94
" 16	4.82	4.45	1.08	6.82	7.63	.89
" 24	7.08	6.62	1.07	10.52	11.53	.91
" 30	10.16	9.27	1.09	15.50	17.72	.87
July 7	12.34	11.53	1.07	22.50	25.76	.87
" 14	18.70	17.76	1.05	34.83	40.00	.87
" 25	27.60	26.15	1.05	49.05	55.70	.88
August 9	42.80	40.60	1.05	67.80	77.41	.87
September 9	77.50	75.00	1.03	95.10	111.42	.85
October 9	138.20	125.00	1.10	145.20	164.00	.88

* Volume determined by displacement.

TABLE IX

HARDNESS OF KIEFFER PEAR AND WAGENER APPLE FRUITS, 1933

DATE OF SAMPLING	KIEFFER PEAR				WAGENER APPLE			
	MEAN	S.D.*	P.E.†	CORRECTED FOR GOV'T. PRESSURE TESTER‡	MEAN	S.D.*	P.E.†	CORRECTED FOR GOV'T. PRESSURE TESTER‡
	lb.			*lb.*	*lb.*			*lb.*
June 10	20.52	1.58	1.06	27.6	20.04	1.75	1.18	27.5
" 16	22.90	1.77	1.19	30.9	19.52	1.06	0.71	26.3
" 24	24.11	0.38	0.25	32.5	20.65	1.78	1.20	27.8
" 30	23.44	1.67	1.12	31.5	22.31	2.14	1.44	30.1
July 7	25.44	1.60	1.07	34.2	20.71	1.42	0.95	28.0
" 14	24.27	1.21	0.82	32.6	20.08	0.87	0.58	27.0
" 25	22.40	0.35	0.23	30.2	18.38	2.43	1.63	24.8
August 9	20.79	1.30	0.87	28.0	16.63	0.80	0.53	22.4
September 9	17.29	1.10	0.74	23.3	12.00	1.55	1.04	16.2
October 9	12.84	0.40	0.26	17.2	10.80	0.79	0.53	14.5

* Standard deviation = $\sqrt{\frac{\text{Summation } X^2}{n} - M^2}$.

† Probable error = S.D. × .6745.

‡ Times factor 1.35 to compare with 5/16 regulation size plunger.

Storage studies

For storage studies, Kieffer fruits were picked at three dates: the first lot on September 29, before the normal picking date for this variety; the second on October 13, about the regular picking time; and the last lot on October 27, later than they are usually harvested. The fruits were picked into baskets and placed immediately in cold storage at 33° F. Samples for chemical analyses were taken monthly during storage. Notes on the condition of the fruits were made at time of sampling.

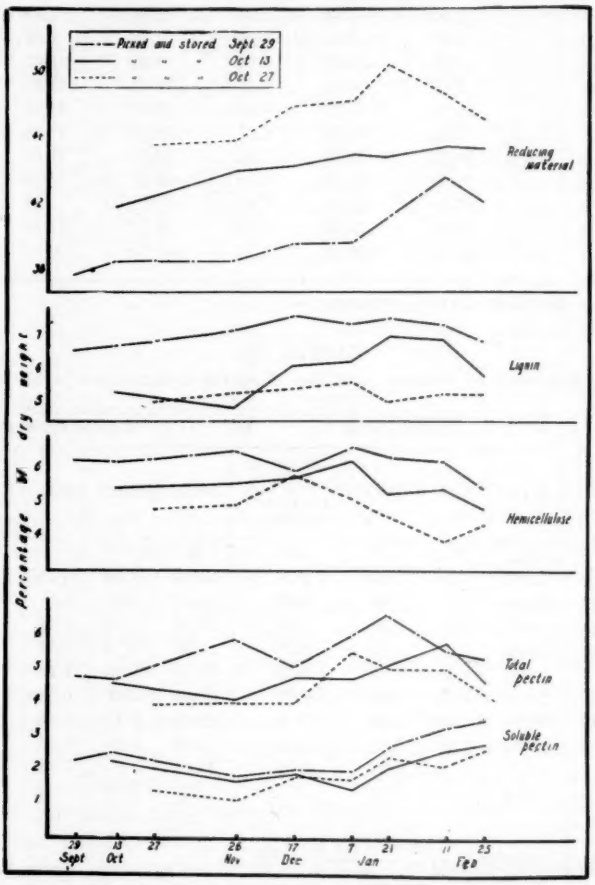


FIG. 7. Percentage change in constituents of Kieffer pear fruits in storage at 33° F., 1931–32.

EMMETT (**13**) in an investigation of changes in pear fruits found that "loss of weight in storage is due chiefly to transpiration." In this study in 1931–1932 the pear fruits showed a loss of 1.5 per cent. of their fresh weight per month. The dry weight concentration of the fruit actually decreased with a corresponding increase in precentage of moisture. In spite of this increase in percentage of moisture the major loss in weight of the fruit is moisture. Thus, paradoxically, the moisture decreases in amount while it is increasing in percentage.

Of the three picking dates represented, October 13 proved to be the best for storage. Determinations of reducing materials, soluble pectin, total pectins, hemicelluloses, and lignin made on these fruits during storage are presented in table VII and figure 7. On the dry weight basis these data show increase in all constituents except hemicellulose during storage. WIDDOWSON found hemicelluloses to decrease in apples in storage. The later-picked fruits had a higher concentration of sugars and lower concentration of lignin, pectins, and hemicelluloses. The intermediate picking was intermediate in all these respects. It may be significant that sugars show a sharp rise followed by a sharp decrease in the early and late pickings. The last picking indicates this break first and this is the inverse order of their keeping quality in storage. This also seems to be the case with total pectins which disappear as pears become overripe and start to break down. EMMETT found this to be the case in Bartlett pears. In general, chemical changes during the ripening and breakdown in storage

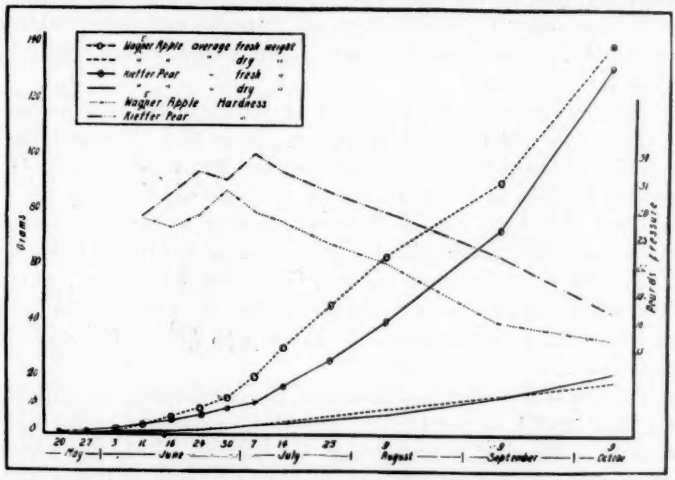

Wagner Apple average fresh weight
" " dry "
Kieffer Pear " fresh "
" " dry "
Wagner Apple Hardness
Kieffer Pear "
Grams
Pounds pressure
20 27 3 11 16 24 30 7 14 23 9 9 9
May June July August September October

FIG. 8. Dry weight, fresh weight, and hardness of Kieffer pear and Wagener apple fruit, 1933.

are similar in pears and apples. The hemicelluloses seem to be the original source of respirable material.

The Kieffer pear fruits picked on October 13 with a pressure of 14.8 pounds skinned or 18.2 pounds unskinned, kept much better in storage than the later or earlier picked fruits. The reducing material content at that time was 42 per cent. of the fruits' dry weight, although as MAGNESS (**19**) states, "differences in chemical composition due to variations in growing conditions are so great in relation to those due to stage of maturity that any picking test based on chemical composition would prove unsatisfactory."

Discussion and conclusions

Assuming that the course of events in the process of lignification occurs as diagrammed, the constituents in order of their complexity would follow the scheme outlined by ONSLOW (**26**). First, some of the sugar becomes oxidized to polyuronic acids, such as galacturonic and glucuronic, which may combine with condensation products of the sugars, such as arabinose and galactose, to form pectic substances. These pectic substances, then, by decarboxylation form five-carbon sugars, such as arabinose, xylose, and some hexoses and uronic acids which together make up the hemicelluloses.

The hemicelluloses may go to lignin, a substance having an uncertain empirical formula. NORRIS and SCHRYVER (**24**) were able to produce some hemicellulose-like material by treating a pectin preparation. CANDLIN and SCHRYVER (**5**), also by treating pectin with alkalis, secured hemicellulose, similar to that isolated from wood in all respects, and some unidentified residues which they state might possibly form combinations with cellulose to produce lignocellulose.

The cellulose seems to be composed of pure glucose and probably is formed directly by condensation of glucose (**27**, p. 67). Lignocellulose has a composition of about 60 per cent. cellulose and 40 per cent. lignin (**7, 27**). Two general theories as to the formation of lignin exist (**16**, p. 49); first, that cellulose of the cell wall is converted directly to lignin or lignocellulose; and second, that materials other than cellulose are lignin precursors.

ONSLOW (**26**, p. 69) supports the first view: "as the cells in plants grow older the walls usually become lignified; that is, part of the cellulose becomes converted to lignocellulose." KÖNIG and RUMP (**18**) also suggest the conversion of cellulose to lignin.

The changes in the relative amounts of the constituents, as indicated in figures 1, 2, 3, and 4, strengthen the hypothesis presented in the lignification diagram. We may consider that the sugars, which the leaves are supplying to the fruits, are being converted to pectins and then to hemicelluloses and finally to lignocellulose. The data show a relative decrease in

sugars during lignification in Kieffer fruit. In the Bartlett fruit with less lignification, the accumulation of sugars is retarded less; and in the apple, with no lignification, no checking of the concentration of sugars occurs. We should expect the pectins, being intermediate products, to be more uniform; and hemicelluloses, because of their greater complexity, to vary more with the end product. This is borne out by their relative concentrations (fig. 2). Associated with the decrease in lignocellulose is an intermediate decrease in hemicelluloses and a sharp increase in sugars. From the graphs showing changes as percentage of the dry weight it is easy to imagine the lignocellulose being broken down to hemicelluloses and then to sugars.

MAGNESS (**19**), referring to his work with Bartlett pears, concludes that "as fruits ripen on the tree, much material other than starch is converted into sugars." MURNEEK (**22**) suggests that in the apple, hemicelluloses are a source of sugar for the maturing fruit. CRIST and BATJER (**7**) suggest a destruction of lignocellulose, and from histological studies, find the clusters of "grit cells" apparently becoming smaller as there is more unlignified tissue between the clusters. These findings seem to support the possibility of a breaking down of the more complex materials to simpler ones in Kieffer pear fruits during the latter part of the growing season.

If the same data are plotted (fig. 5) as absolute amounts per fruit during the growing season, it becomes difficult to imagine any of the constituents breaking down. The data show clearly an increase in every fraction. Hemicellulose changes are almost identical with lignin changes (table III, fig. 2) and would, if presented as absolute amounts per fruit, show the same increase during the growing season. With these particular data, the possibility of hemicellulose supplying sugar to the maturing Kieffer pear fruit is not supported. The case may be quite different in the apple, however, as an inspection of figure 6 reveals a slight decrease during the latter part of the growing season in total amounts of lignin and cellulose. As the absolute amount of lignocellulose in the pear fruit does not decrease, but actually increases, the destruction of lignocellulose (**7**) could be accounted for by its being formed in new parts of the fruit, as nearer the periphery, faster than it is destroyed in the more concentrated areas. The writer doubts that actual destruction of lignocellulose occurs. An apparent decrease in size of the "grit cell" clusters may be due to the clusters being pushed farther apart as the fruit increases in size, due to increase in size of individual cells in the latter part of the growing season, thus distributing the stone cells over a greater area.

It therefore becomes apparent that there is no basis for the support of the supposition that lignocellulose is being converted over to sugars or

to any other material. However, it does seem quite probable that the building up of lignocellulose is through these intermediate materials.

It is evident, therefore, that the amount of grit cells of pears depends principally on the extent of grit cell formation during early stages of the formation of the fruit and apparently is not reduced by changes taking place in the grit aggregation during the latter part of the growing season or during ripening. This in turn means that the process is not likely to be materially influenced by cultural or handling practices and bears out the suggestion of CRIST and BATJER that only through his choice of varieties does the pear grower have any considerable control over this more or less objectionable characteristic of pear fruits. A distinction between absolute amount and apparent grittiness is evident when the fruit becomes soft, as when allowed to ripen on the tree, permitting the grits to separate easily from the pulp and making them more noticeable. A fruit ripening on the tree is increasing more rapidly in substances other than grit cells, although it is actually increasing slightly in absolute amounts of grit cells. In apparent grittiness it is increasing greatly. This accounts for the popular impression that "grittiness" increases when pear fruits are allowed to ripen on the tree.

Summary

1. Existing data (and the first two years' results of this study) showed such a tremendous decrease of the percentage of lignocellulose (stone cells) accompanied by an equally great increase of reducing materials, in maturing Kieffer pear fruits, that it suggested that part of the lignocellulose had been converted to reducing substances. Investigations of these changes in the Bartlett pear, a fruit in which lignocellulose occurs in smaller quantities, showed that as a percentage of the dry weight the lignocellulose began to decrease, with a corresponding increase in reducing substances, about 10 days earlier than in the Kieffer. Similar studies of the Wagener apple, a fruit which contains very little lignocellulose, indicated decreases of lignocellulose and accumulation of reducing substances occurring in the first samples taken soon after petal fall. These results strengthened the supposition that lignocellulose may be converted to reducing materials.

2. During the growing season of 1933, changes in absolute amounts of these materials in the Kieffer pear and Wagener apple were determined. These determinations show that there was no actual decrease in lignocellulose, but because of the great increase of alcohol soluble materials, the *percentage* of lignocellulose decreased rapidly. The findings in 1933 indicate that lignified tissue does not break down to form less complex materials in these fruits during growth.

3. Calculated changes in composition may be misleading when presented as percentages. Total "grit" in pear is not reduced during ripening, but "grittiness" is masked by the increased amounts of other constituents of the fruit.

4. There is evidence, however, that the sugars are built up, through the compounds studied, to lignified tissue.

5. In storage, hemicelluloses decreased more than any other constituents; this suggests that these materials may be the source of respirable substances for the fruit after its removal from the tree.

6. The modified SHAFFER-HARTMANN titration method, employed for determining amounts of reducing material during the first two years' study, was found to give higher values on young pear and young apple fruit extracts than the amounts of copper reduced warrants. The high proportion of skin to flesh in the samples taken when the fruits were small is so closely associated with these unwarranted high values that adsorption of the iodine reagent by some material in the skin is indicated.

7. The suggestion of CRIST and BATJER that the grower has little control, other than variety selection, over the "grittiness" of his pear fruits, is supported.

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STUDIES OF THE EFFECT OF ARTIFICIAL WIND ON GROWTH AND TRANSPIRATION IN *HELIANTHUS ANNUUS*

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(WITH TEN FIGURES)

Introduction

Although considerable attention has been given to the influence of wind on transpiration, the results that have been obtained are very conflicting. Practically all control experiments with this factor have been in the region of very low velocities. Knight (**12**) found that a wind velocity of 7 meters per minute (0.26 miles per hour) caused an increase in the rate of transpiration of about 50 per cent. Stålfelt (**21**), using single detached leaves suspended in an analytical balance case, obtained a very rapid rise with wind up to a velocity of 10 meters per minute, a slower increase from there to 30 m./min., but no further increase as the wind rose to 60 m./min. (2.24 miles per hour). This maximum increase was 140 per cent. of the value with no wind. Firbas (**8**), who employed cut shoots under a wind velocity of 3.75 m./sec., obtained similar results with low rates of transpiration, but with high rates (shoots in direct sunlight) wind actually caused a decrease. Seybold (**18, 19, 20**) concluded that wind has no influence on stomatal transpiration but only on the cuticular component, the result being that only those plants with a high cuticular transpiration, namely hydrophytes, showed an appreciable increase in the transpiration rate due to the action of wind.

Observations on water losses of plants under natural conditions have also yielded conflicting results. Copeland (**6**) found an increase in the transpiration rate of cocoanut palm in full sunlight of about 100 per cent. with a wind velocity estimated at 5 miles per hour. Others (**4, 9**) have noted an increase with velocities up to 8 miles per hour, but a retardation with higher velocities. Wilson (**22**) observed that the rate of transpiration of some Australian plants was checked when the wind velocity rose to 20 miles per hour. Briggs and Shantz (**2, 3**), however, concluded from their studies of the correlation between environmental factors and transpiration rates for the growing season that only from 2 to 6 per cent. of the water loss could be attributed directly to the action of wind.

Nearly all the control work in this field has been with cut shoots or leaves, which rarely give dependable results, while that with rooted plants has for the most part been under natural conditions with no attempt to control the wind velocity. A combination of these methods has been utilized

in the present work by growing plants in cans but under otherwise natural conditions and producing artificial wind by means of large fans.

These fans have also been employed in a study of the effect of continuous wind upon plant growth. Although the depressing effect of wind has long been recognized, very few measurements of a quantitative nature have been made. BLANCHARD (1) compared the growth and yield of two orchards of Eureka lemons and found that the factor of improvement in yield due to protection from wind was 5 to 7 and in size of trees about 3. FINNELL (7) has grown marigolds under a constant wind velocity of 15 miles per hour and found that it caused a reduction of about 50 per cent. in dry weight of material produced.

I. Effect of wind on transpiration; short time series

METHODS

For all series, plants of *Helianthus annuus* were grown from seed in water tight, cylindric, galvanized iron cans 8 in. in diameter and 10 in. in height. Each of these was fitted with a removable lid that had in its center a circular opening 2 in. in diameter. The soil used was a good loam of as nearly uniform texture and moisture as was feasible. The holard was set at approximately 65 per cent. of saturation (or 28 per cent. of the dry weight of the soil), and the total amount of water in each can was kept within a few per cent. of this value throughout the growing period as well as during the test series. When necessary, water was added through a glass tube extending into a layer of gravel about 1 in. thick in the bottom of the can. When the plants were 6 to 8 weeks old, the containers were sealed by adding a layer of sand about $\frac{3}{4}$ in. thick on top of the soil, filling the opening in the lid with non-absorbent cotton, and corking the glass tube. The efficiency of this seal was tested by means of controls without plants. Weighings made on a torsion balance to the nearest gram during the time the experiments were in progress showed that these containers seldom lost a detectable amount.

Wind was produced by fans taken from Pierce-Arrow and Packard motor cars, mounted on specially constructed supports, and driven by $\frac{1}{3}$ h.p. induction motors. The wind velocity was regulated partly by pulley ratio and partly by adjusting the distance of the fans from the plants. Velocities from about 0.5 to 25 miles per hour were obtainable with this installation and measurements were made by means of a small fan type anemometer.

In all series, two sets of four plants each were used, one serving as a test set, the other as a control. The two sets were selected from a large group in order that they might be as nearly alike as possible. To obtain conditions sufficiently calm for these experiments, it was necessary to work inside the greenhouse, since there is practically always some movement of

TABLE I

TRANSPIRATION RATES IN GRAMS PER SQUARE DECIMETER PER HOUR FROM SERIES TAKEN INSIDE GREENHOUSE

DATE		NO WIND		WIND = 0.8 M.P.H.					NO WIND		WIND
5/8/35	Time interval	9:30–10:30	10:30–11:30	11:30–12:00	12:00–12:30	12:30–1:30	1:30–2:30	2:30–3:30	3:30–5:00	5:00–8:00	8:00 P.M. 8:30 A.M.
	T_a*	0.512	0.539	0.678	0.892	1.08	1.10	1.33	0.787	0.308	0.152
	T_b	0.499	0.544	0.859	1.23	1.38	1.47	1.81	0.727	0.272	0.189
	T_b/T_a	0.98	1.01	1.25	1.38	1.98	1.34	1.36	0.92	0.88	1.24
		NO WIND		WIND = 2.1 M.P.H.					NO WIND		WIND
5/9/35	Time interval	8:30–9:30	9:30–11:30	11:30–12:00	12:00–12:30	12:30–1:30	1:30–2:30	2:30–3:30	3:30–5:00	5:00–9:30	6:30 P.M. 8:30 A.M.
	T_a*	0.535	0.605	0.757	0.854	0.850	0.542	0.516	0.445	0.160	0.108
	T_b	0.578	0.593	1.05	1.25	1.16	0.637	0.711	0.548	0.146	0.136
	T_b/T_a	1.08	0.98	1.39	1.46	1.37	1.18	1.38	1.23	0.91	1.26
		NO WIND		WIND = 5.5 M.P.H.					NO WIND		WIND
/10/35	Time interval	8:30–10:15	10:15–11:30	11:30–12:00	12:00–12:30	12:30–1:30	1:30–2:30	2:30–3:30	3:30–5:15	5:15–8:30	8:30 P.M. 8:00 A.M.
	T_a*	0.344	0.707	0.972	1.20	0.682	0.717	1.09	0.595	0.213	0.127
	T_b	0.340	0.710	1.57	1.59	0.856	1.01	1.50	0.540	0.195	0.159
	T_b/T_a	0.99	1.00	1.61	1.32	1.27	1.41	1.38	0.91	0.91	1.25
		NO WIND		WIND = 9.5 M.P.H.					NO WIND		WIND
/11/35	Time interval	8:00–9:40	9:40–11:40	11:40–12:00	12:00–12:30	12:30–1:30	1:30–2:30	2:30–3:30	3:30–5:30	5:30–8:30	8:30 P.M. 7:30 A.M.
	T_a*	0.590	1.52	2.24	2.29	2.63	2.34	1.64	0.676	0.161	0.122
	T_b	0.572	1.57	4.44	3.21	3.98	3.16	2.25	0.568	0.136	0.179
	T_b/T_a	0.97	1.03	1.98	1.40	1.51	1.35	1.37	0.84	0.84	1.47

* T_a and T_b are the transpiration rates of the two sets of plants *a* and *b*, while their ratio is given by T_b/T_a. Set is the control and has no wind at any time, while *b* is the test set and has wind during the periods indicated.

TABLE I—(*Continued*)

TRANSPIRATION RATES IN GRAMS PER SQUARE DECIMETER

DATE		NO WIND			WIND = 16 M.P.H.				
	Time interval	8:30–9:30	9:30–10:30	10:30–11:50	11:50–12:05	12:05–12:30	12:30–2:00	2:00–2:30	2:30–3:30
5/14/35	T_a*	1.05	0.95	1.16	1.18	0.99	1.11	0.86	0.75
	T_b	1.01	1.07	1.07	2.81	1.23	1.53	1.29	1.18
	T_b/T_a	0.96	1.13	0.92	2.38	1.24	1.38	1.50	1.57
		NO WIND		WIND = 2.6 M.P.H.			NO WIND		
	Time interval	9:30–10:10	10:10–10:48	10:48–11:03	11:03–11:38	11:38–12:41	12:41–1:34	1:34–2:38	
5/15/35	T_a	0.99	0.95	0.79	0.80	0.69	0.79	1.22	
	T_b	1.05	0.89	1.41	0.96	0.91	0.71	1.19	
	T_b/T_a	1.06	0.94	1.78	1.20	1.32	0.90	0.97	
		NO WIND	WIND = 0.7 M.P.H.			NO WIND			
	Time interval	8:00–9:44	9:44–9:59	9:59–10:37	10:37–11:34	11:34–11:49	11:49–1:13	1:13–4:59	4:59–5:45
5/16/35	T_a	1.15	1.97	2.08	2.49	2.89	2.85	2.13	1.10
	T_b	1.20	2.44	2.44	3.16	2.66	2.66	2.06	1.28
	T_b/T_a	1.04	1.24	1.17	1.27	0.92	0.93	0.97	1.16

* T_a and T_b are the transpiration rates of the two sets of plants *a* and *b*, while their ratio is wind during the periods indicated.

air in the open. The greenhouse measures 39 ft. × 16 ft., and hence is quite large enough for the purpose. Stomatal openings were determined at regular and frequent intervals during each series by means of the well known alcohol method (LLOYD, **14**). Leaf areas in square decimeters were determined by multiplying the product of the length and width of each leaf in centimeters by the factor 0.0134 (CLEMENTS and GOLDSMITH, **5**).

RESULTS

The transpiration measurements from seven representative series are given in table I, in which T_a denotes the rate of transpiration of the control set of plants in grams per square decimeter per hour, T_b that of the test set, and T_b/T_a the ratio of the rate of water loss of *b* to that of *a*. These results are shown graphically in figures 1 and 2, which portray the transpiration rate of the test set in percentage of that of the control. Each series began in the morning with one or more periods during which neither set had any wind, followed by several hours of wind, a second check period of two, and

TABLE I—(*Continued*)

PER HOUR FROM SERIES TAKEN INSIDE GREENHOUSE

No wind		Wind	No wind				
3:30–6:00	6:00–8:45	8:45 P.M. 8:50 A.M.	8:50 A.M. 10:15 A.M.				
0.524	0.133	0.151	0.95				
0.510	0.177	0.302	0.94				
0.97	1.33	2.00	0.99				

Wind = 5.4 m.p.h.				No wind			
2:38–2:53	2:53–3:23	3:23–3:53	3:53–5:18	5:18–5:33	5:33 P.M. 8:00 A.M.		
1.13	1.23	0.92	0.91	0.70	0.170		
2.14	1.55	1.05	1.32	0.59	0.166		
1.89	1.26	1.14	1.45	0.84	0.98		

Wind = 16 m.p.h.					No wind		
5:45–6:00	6:00–6:15	6:15–6:30	6:30–8:15	8:15 P.M. 8:28 A.M.	8:28–8:44	8:44–9:03	9:93–10:06
1.04	0.70	0.475	0.219	0.250	1.44	1.54	1.96
2.08	0.92	0.703	0.386	0.384	1.10	1.44	1.75
2.00	1.31	1.48	1.76	1.54	0.76	0.93	0.89

given by T_b/T_a. Set *a* is the control and has no wind at any time, while *b* is the test set and has

finally usually wind again during the night. For all series, the losses of the two sets during the initial check periods were the same within a few per cent., but when the wind was turned on the test set, its transpiration rate invariably rose with respect to that of the control, the increase depending on the wind velocity.

For the low velocity winds, up to about 2 miles per hour, the transpiration rate increased from 20 to 30 per cent., and this value was maintained approximately constant during the entire time the wind was turned on. For higher velocities, however, there was a comparatively high initial rise lasting no more than 15 minutes and in many cases probably less, followed by a much lower value, after which there was usually a gradual increase. This high initial rise increased with rising wind velocity, being 78 per cent. for 2.6 m.p.h., 89 for 5.4, 98 for 9.5, and 138 for 16 m.p.h. These values are considerably higher than the averages for the entire periods that the wind was acting. These latter values are given in table II, for both the day and night periods, and the daytime ones are shown graphically in figure 3. The

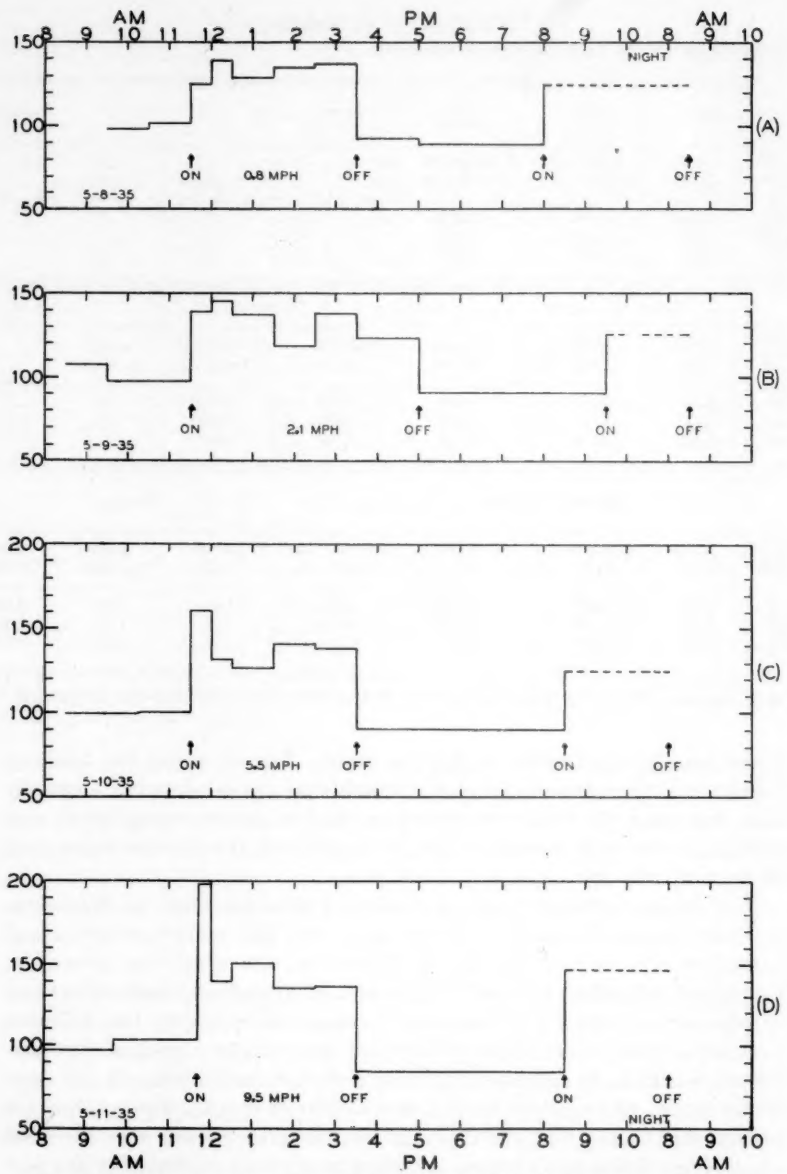

FIG. 1. Results from four of the short time series taken inside the greenhouse. The ordinate represents the transpiration rate of the test set in percentage of that of the control, while the abscissa indicates time of day. Values obtained during the night are shown by the dashed lines.

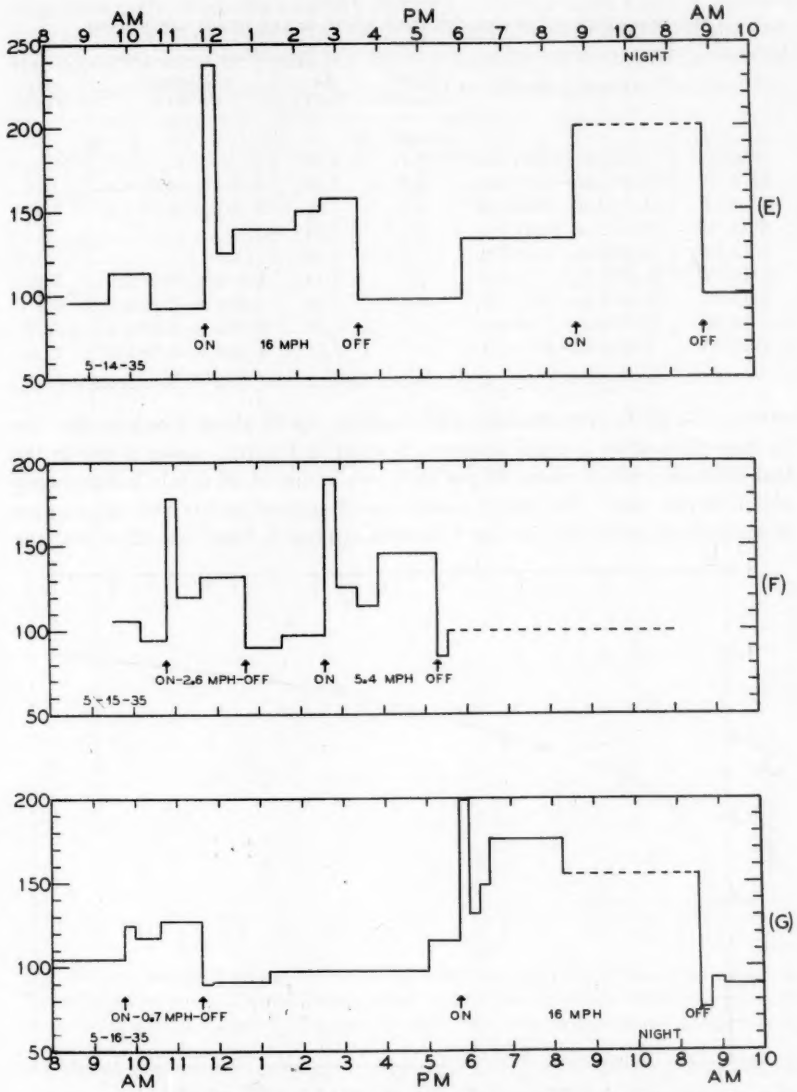

FIG. 2. Results from three others of the short time series. Ordinates are the same as in figure 1.

TABLE II

GENERAL SUMMARY OF RESULTS FROM SERIES TAKEN INSIDE GREENHOUSE

DATE	DAYTIME INTERVAL	WIND VELOCITY	AV. T_b/T_a	NIGHTTIME INTERVAL	AV. T_b/T_a
		m.p.h.			
5/16/35	9:44 A.M.–11:34 A.M.	0.7	1.23	.....	.....
5/ 8/35	11:30 A.M.– 3:30 P.M.	0.8	1.32	8:00 P.M.–8:30 A.M.	1.24
5/ 9/35	11:30 A.M.– 5:00 P.M.	2.1	1.34	9:30 P.M.–8:50 A.M.	1.26
5/15/35	10:48 A.M.–12:41 P.M.	2.6	1.34	.....	.....
5/15/35	2:38 P.M.– 5:18 P.M.	5.4	1.40	.....	.....
5/10/35	11:30 A.M.– 3:30 P.M.	5.5	1.38	8:30 P.M.–8:00 A.M.	1.25
5/11/35	11:40 A.M.– 3:30 P.M.	9.6	1.46	8:30 P.M.–7:30 A.M.	1.47
5/14/35	11:50 A.M.– 3:30 P.M.	16	1.50	8:45 P.M.–8:50 A.M.	2.00
5/16/35	5:45 P.M.– 8:15 P.M.	16	1.71	8:15 P.M.–8:28 A.M.	1.54

ratio of T_b to T_a rises rapidly with velocity up to about 2 m.p.h., but the increase thereafter is much slower. A wind of 1 m.p.h. causes a rise in the transpiration rate of about 30 per cent., while one of 16 m.p.h. induces only about 50 per cent. The night values are of approximately this same order of magnitude, although the low velocities appear to have less effect and the

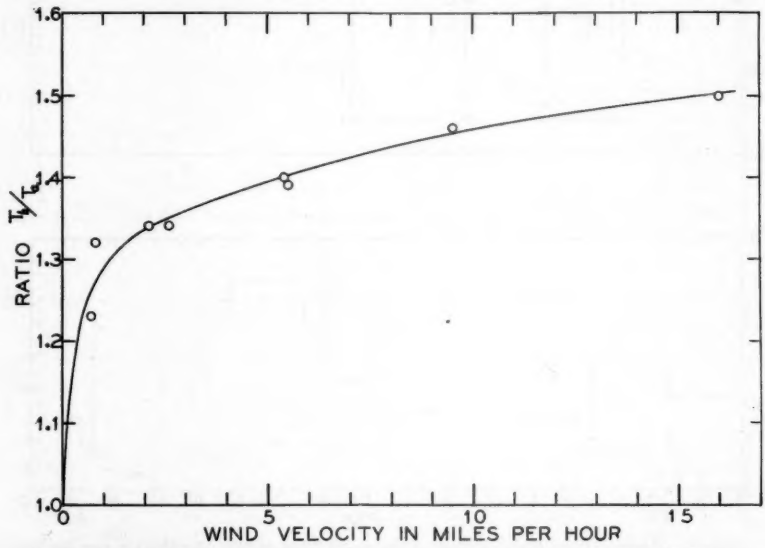

FIG. 3. The ratio of the transpiration rate of the test set (T_b) to that of the control (T_a) represented as a function of wind velocity. The values are all averages for from 2 to 4 hours of wind during the daytime hours.

high ones more effect than during the day. Although the *relative* increase in transpiration rate during the night is nearly the same as during the day, the *actual* increase in grams per square decimeter per hour is much less. This effect is shown graphically in figure 4 by the data taken May 11, 1935.

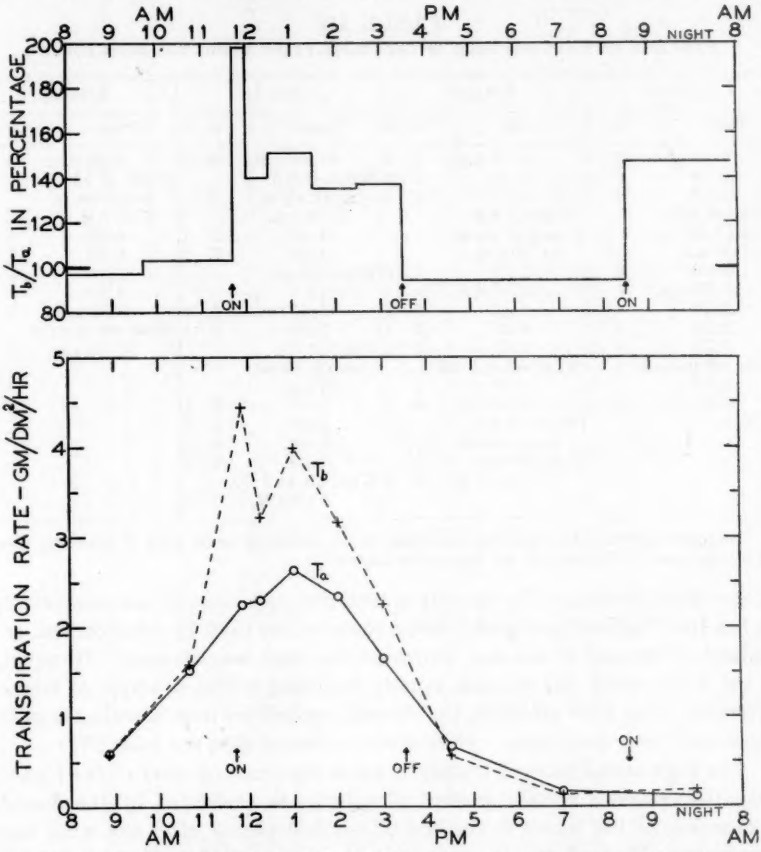

FIG. 4. Results from the short time series of May 11, 1935, with a wind velocity of 9.5 miles per hour. The actual transpiration rates of the test and control sets are shown in the lower part as T_b and T_a respectively, while their ratio in percentage is shown above.

The lower section of figure 4 shows the actual transpiration rates of the two sets of plants and the upper their ratio. Wind in this case caused a rise of about 46 per cent. both day and night, although the actual increase in grams per square decimeter per hour during the day was about 20 times as great as during the night.

The behavior of the stomata during the various series depended on the wind velocity, currents below about 2 m.p.h. having no observable effect, those above this value usually showing a distinct influence. The stomatal openings for some of the series, given in table III, show the action typical

TABLE III

STOMATAL OPENINGS FOR SOME OF THE SERIES TAKEN INSIDE THE GREENHOUSE

5/10/35			5/11/35			5/15/35			5/16/35		
TIME	*a*	*b*	TIME	*a*	*b*	TIME	*a*	*b*	TIME	*a*	*b*
8:30 A.M.	5	5	8:00 A.M.	5	5	10:50 A.M.	5	5	5:45 P.M.	3	3
10:30	5	5	9:45	5	5	Wind of 2.6			Wind of 16		
11:30	5	5	11:30	5	5	m.p.h on at			m.p.h. on at		
Wind of 5.5			Wind of 9.5			10:50 A.M.			5:45 P.M.		
m.p.h on at			m.p.h. on at			11:10	5	1	6:00	3	1
11:30 A.M.			11:40 A.M.			11:45	5	2	6:15	3	1
12:00	5	1	12:00	5	0	Wind off at			6:30	3	1
12:30 P.M.	5	1	12:30 P.M.	5	1	12:42			8:20	0	0
1:30	4	1	1:30	5	1	12:45 P.M.	5	2	8:30 A.M.	5	3
2:30	4	1	2:30	5	1	2:35	5	4	Wind off at 8:30		
3:30	4	1	3:30	5	1	Wind of 5.4			10:15 A.M.	5	5
Wind off at 3:30			Wind off at 3:30			m.p.h. on at					
5:15	1	3	5:30	2	1	2:38 P.M.					
8:15 P.M.	0	1	8:30 P.M.	0	0	3:00	5	1			
			Wind of 9.5			3:30	5	1			
			m.p.h. on at			4:00	4	1			
			8:30 P.M.			5:20	3	2			
			7:30 A.M.	4	2	Wind off at 5:20					
						8:00 A.M.	5	5			

*Stomatal openings are graded according to an arbitrary scale with 0 denoting closed and 5 wide open; *b* is the test set and *a* the control.

of the effect of wind. The stomata closed, although usually not completely, in less than half an hour and in some cases in less than 15 minutes, and remained so the rest of the day, provided the wind was blowing. However, if the wind ceased, the stomata usually reopened within a couple of hours. When the wind blew all night, the stomata opened the next morning to only about half their maximum. (For other pertinent data see table IV.)

The high initial rates of transpiration at the onset of wind of the higher velocities caused a certain amount of wilting, as evidenced by the flaccid appearance of the leaves at the end of the first period after the wind was turned on. This effect was evident in the series of May 15 with the 5.4 m.p.h. wind, that of May 11 with the 9.5 m.p.h. wind, and those of May 14 and 16 with the 16 m.p.h. wind, but in none of the others. In all cases this effect appeared in about 15 minutes and vanished in about half an hour, and in no case was it very marked. Wind of 16 m.p.h. caused slight bruising around the edges of some of the leaves and in places where they folded over or touched each other; however, this effect was not noticeable with lower velocities.

TABLE IV

VALUES OF AIR TEMPERATURE IN DEGREES FAHRENHEIT AND RELATIVE HUMIDITY IN PERCENTAGE FOR THE SERIES TAKEN INSIDE GREENHOUSE

5/8/35	Time	9:30	11:30	12:30	1:30	2:30	3:30	5:15	8:20	
	Air temp.	74	75	83	86	91	87	78	67	
	Rel. hum.	58	51	47	47	40	41	47	56	
5/9	Time	8:30	9:30	11:30	12:30	1:30	2:30	3:30	5:15	9:30 P.M.
	Air temp.	72	76	77	82	80	77	77	72	65
	Rel. hum.	59	53	54	50	52	52	52	57	68
5/10	Time	8:30	10:15	11:30	12:30	1:30	2:45	3:30	5:15	8:30
	Air temp.	65	73	82	80	76	81	83	78	62
	Rel. hum.	68	57	51	52	55	51	50	53	64
5/11	Time	9:45	11:45	12:30	1:30	1:55	2:30	3:30	5:30	8:30
	Air temp.	84	99	102	108	100	95	84	72	63
	Rel. hum.	51	36	36	33	37	30	37	43	57
5/14	Time	8:30	9:30	10:30	12:00	12:30	2:00	3:30	6:15	8:45
	Air temp.	69	70	78	80	81	81	78	70	61
	Rel. hum.	59	55	46	45	47	47	49	53	63
5/15	Time	9:00	10:20	11:15	12:45	1:45	2:45	3:45	5:30	
	Air temp.	74	80	81	78	86	84	81	84	
	Rel. hum.	58	45	49	51	44	43	44	46	
5/16	Time	8:00	10:00	11:30	12:00	87	86	80	64	80
	Air temp.	72	85	102	95	37	33	39	58	47
	Rel. hum.	55	41	36	31	1:20	5:20	6:05	8:20	8:30 A.M.

The very high initial rate of transpiration that takes place at the onset of wind in the higher range of velocities apparently causes a reduction in the sap content of the leaves as shown by their slightly flaccid appearance at this time and a consequent closure of stomata, both of which factors doubtless play a part in the reduction of the relative transpiration rate that occurs during the second weighing interval. Following this second period, the flaccidity disappears and there usually occurs a rather slow increase in the relative transpiration rate, which continues until the wind ceases. The recovery of turgor in the leaves and the fact that the stomata remain at about the same degree of opening during this period of gradual increase indicate that the rise must be due to a gradual restoration of the sap content of the leaves with resultant increase of transpiration. The fact that this gradual increase exists at all indicates that the closure of the stomata retards transpiration sufficiently to permit recovery of the sap content of the leaves.

On the other hand, an indication that the stomata do not always play a controlling part in regulating transpiration is obtained from the relative transpiration rate immediately after the wind stops. Though there is at this time practically always a reduction in the rate of the test set below that of the control, it is rarely more than 20 per cent., in spite of the fact that the stomatal opening may be considerably less. In this connection it is important to note that even in cases when the stomata are open, a drop of about 10 per cent. occurs, due probably in these cases to the lower sap content of the leaves. This view is supported by the fact that the relative influence of wind on the rate of water loss during the night, when the stomata are closed, is much the same as during the day. This means either that wind has practically the same effect on the cuticular as on the stomatal component of transpiration, or that stomata may sometimes play only a small part in the regulation of transpiration. This latter view is supported by the work of other investigators (**10, 13, 15, 16**).

Comparison of the data from different series indicates that the closure of stomata by wind is not entirely due to the water relations, but at least partly to mechanical action. This is well illustrated by comparison of the stomatal behavior of the test set on May 10 (wind of 5.5 m.p.h.) with that of the control on May 11. In the former case, the stomata closed and remained so while the wind continued, but in the latter they remained wide open, even though the transpiration rate throughout the series was nearly twice as great. A direct test of this assumption is difficult, since it is hard to shake a leaf without increasing its transpiration, and it is also difficult to prevent wind shaking the leaf. This effect is in agreement with the work of KNIGHT (**11**) and LOFTFIELD (**16**), who found that the stomata of some plants are sensitive to the shock of handling the leaves.

Discussion

The assumption that the high initial rise in the transpiration rate that occurs at the onset of winds of velocities greater than about 3 m.p.h. lasts probably less than 15 minutes is supported by the experimental data. The effect is illustrated best by the series of May 16 with the 16 m.p.h. wind. In this series, the transpiration rate for the first 15 minutes after the wind started was 2.08 grams per square decimeter per hour, representing an increase of 100 per cent. with respect to the control, while for the next period, also 15 minutes, the rate was 0.92, or an increase of only 31 per cent. with respect to the control. This large decrease in the relative transpiration rate during the second period indicates that at the beginning of the initial period the increase in the rate of the test set must have been much greater than 100 per cent. and at the end smaller. This assumption is further supported by the fact that at the end of the initial 15-minute period, the leaves showed some evidence of flaccidity even though the transpiration rate was only 2.08 gm./dm.^{2}/hr., while during the period 12: 30–1: 30 on May 11 the rate of transpiration of the test set was 3.98 with no evidence of flaccidity. This sort of action appears to be explainable only by the assumption that the transpiration rate must be very high for the first few minutes of the initial period of wind with a subsequent rapid decrease. It is unfortunate that the methods used in these experiments did not permit a direct test of this assumption, but 15 minutes was the shortest time interval that could be employed without too great sacrifice of accuracy in weighings.

When the stomata do respond to the action of wind, they close partially or completely, as the case may be, in less than half an hour, and for the high velocities in less than 15 minutes. The lowest velocity of wind that will induce closure of stomata seems to be somewhere between 2 and 3 m.p.h., although some series have been taken in which wind of as high as 5 m.p.h. had no noticeable effect on the stomata. In these, however, there was no indication of the very high initial rise in the relative transpiration rate that occurs in the series given here with the same wind velocity, although the average increase in the rate for the entire period of wind agreed very closely. The range between 2 and 5 m.p.h. appears to be critical in that wind of these velocities will sometimes close stomata, sometimes not, apparently depending upon some factor or factors to which it cannot at present be assigned. Below this range, wind has not been observed to affect the stomata, but above it invariably does so.

II. Effect of wind on growth and transpiration; long time series

Methods

For these experiments, plants of *Helianthus annuus* were grown from seed in cans of the type described above, with three or four seeds in each.

When the plants had reached the first leaf stage, they were selected for uniformity of height and leaf size, leaving only one in each can. The cans were then sealed with sand and cotton, and placed in wooden boxes $2 \times 4 \times 1$ ft. deep, sunk in the ground with their tops level with the surface. These boxes were covered with rather closely fitting lids of masonite in order to prevent the large variations in soil temperature that would occur if the cans were exposed directly to the sun's rays. Four of these boxes were used with six phytometers and one check can in each. One set served as a control with only the natural wind, while the other three had artificial wind of about 5, 10, and 15 miles per hour produced by the fans described above. These fans measure approximately 20 to 22 inches in diameter, so that the stream of air was quite wide enough to cover the entire set of plants throughout the growing period.

The water content was maintained constant within a few per cent. and a record of water used was kept. Measurements of leaf areas and diurnal variations in transpiration rates and stomatal openings were made at intervals during the growing period. When the plants had become as large as the wind current would cover, the series was terminated, final measurements of leaves and stems were made, and the plants were dried in a large oven to obtain their dry weights (**17**).

Of the four series conducted, two were in the fall and two in the spring. Series I began with the planting on September 25, 1933, and ended November 17; the second ran from March 13, 1934, to May 2; the third started September 15, 1934, and ended October 26; and the fourth lasted from February 26, 1935, to April 25.

Results

The average leaf areas of plants taken at intervals during series I are given in table V and are shown graphically in figure 5. The four curves, A, B, C, and D, correspond to the four sets of plants, A, B, C, and D, respec-

TABLE V

AVERAGE LEAF AREAS OF PLANTS IN SQUARE DECIMETERS AT INTERVALS DURING THE GROWING PERIOD; SERIES I

	DATE					
A	1.16	3.58	10.70	19.00	22.20	46.5
B	1.17	3.78	10.20	18.30	21.20	44.5
C	0.83	2.20	5.50	10.90	12.90	26.8
D	0.70	1.92	3.63	6.33	7.33	13.9

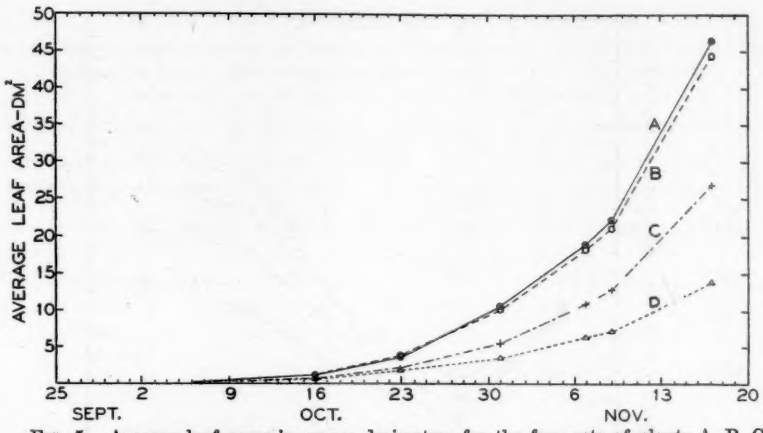

FIG. 5. Average leaf areas in square decimeters for the four sets of plants A, B, C, and D with wind velocities of about 0, 5, 10, and 15 miles per hour respectively, determined at intervals during series I.

TABLE VI

WEEKLY AVERAGE TRANSPIRATION RATES DURING THE GROWING PERIOD FOR SERIES IV

PERIOD	SET NO.	AVERAGE TRANSPIRATION RATE	
		GM./DM.²/24 HR.	PERCENTAGE OF CONTROL
3/20/35	A (control)	11.4	100
to	B	11.4	100
3/27	C	11.0	96
	D	10.9	96
3/27	A	5.94	100
to	B	6.40	108
4/3	C	7.09	119
	D	7.88	133
4/3	A	8.04	100
to	B	9.62	120
4/10	C	10.8	134
	D	10.3	128
4/10	A	11.3	100
to	B	14.0	124
4/17	C	15.9	141
	D	17.5	155
4/17	A	12.6	100
to	B	15.0	119
4/24	C	16.8	133
	D	18.3	145

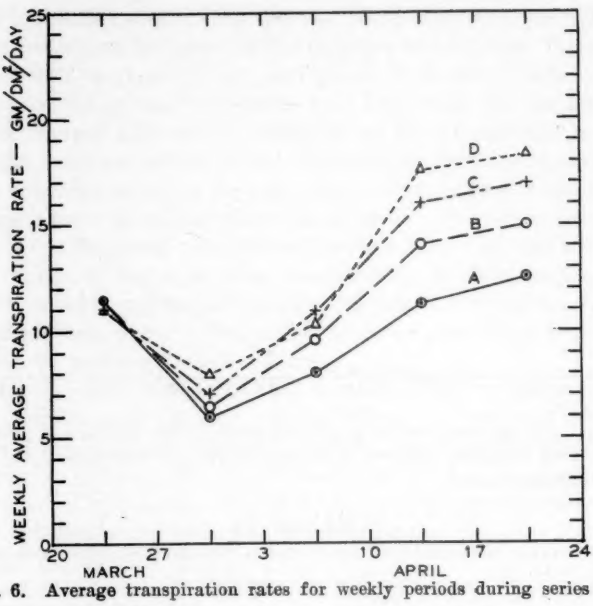

FIG. 6. Average transpiration rates for weekly periods during series IV.

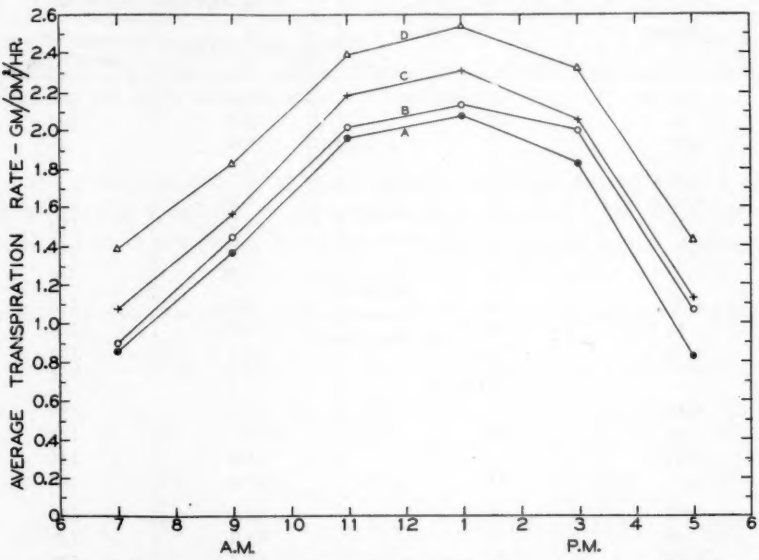

FIG. 7. Daily march of transpiration of the four sets of plants near the end of series II. Data taken May 2, 1934, at which time the plants were 7 weeks of age.

TABLE VII

DAILY MARCH OF TRANSPIRATION OF PLANTS GROWN UNDER CONTINUOUS WIND; SERIES II. DATA TAKEN MAY 2, 1934, AT WHICH TIME THE PLANTS WERE 7 WEEKS OF AGE. VALUES GIVEN ARE IN GRAMS PER SQUARE DECIMETER PER HOUR

SET NO.	6–8	8–10	10–12	12–2	2–4	4–6	6–6 (NIGHT)
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
A	0.86	1.37	1.96	2.08	1.83	0.83	0.071
B	0.90	1.45	2.02	2.14	2.00	1.07	0.005
C	1.08	1.57	2.10	2.31	2.06	1.13	0.191
D	1.39	1.83	2.39	2.54	2.32	1.43	0.306

tively, with wind velocities of approximately 0, 5, 10, and 15 miles per hour. The average transpiration rates for weekly periods during series IV were obtained from the average leaf areas and total transpiration during those periods; they are given in table VI and are shown graphically in figure 6.

TABLE VIII

RESULTS FROM THE FOUR SERIES

SERIES	SET NO.	AV. LEAF AREA	AV. STEM HT.	AV. STEM DIAM.	AV. DRY WT.	AV. TOTAL TRANSPIRATION	AV. WATER REQUIREMENT	AGE OF PLANTS	NO. PLANTS PER SET
		dm.^{2}	*cm.*	*mm.*	*gm.*	*gm.*	*gm./gm.*	*days*	
	A	46.5	62	14.7	33.5	7520	224		5
I	B	44.5	63	15.0	29.0	6870	237	53	6
	C	26.8	40	11.4	18.5	4960	268		6
	D	13.9	33	10.3	11.2	3210	286		6
	A	28.0	63	13.3	40.0				6
II	B	18.7	47	12.2	28.6			50	4
	C	18.0	45	11.7	26.6				5
	D	13.5	44	11.6	22.4				6
	A	37.0	53	13.9	21.3	4730	222		6
III	B	31.8	42	12.5	19.1	4320	226	41	5
	C	15.5	33	9.8	10.2	2770	271		6
	D	8.5	23	8.6	6.2	1760	284		6
	A	15.4	48	11.3	16.5	2640	160		6
IV	B	14.0	48	11.2	15.8	2765	175	58	6
	C	12.9	47	11.0	12.2	2515	206		6
	D	9.3	32	9.6	7.1	1770	249		5

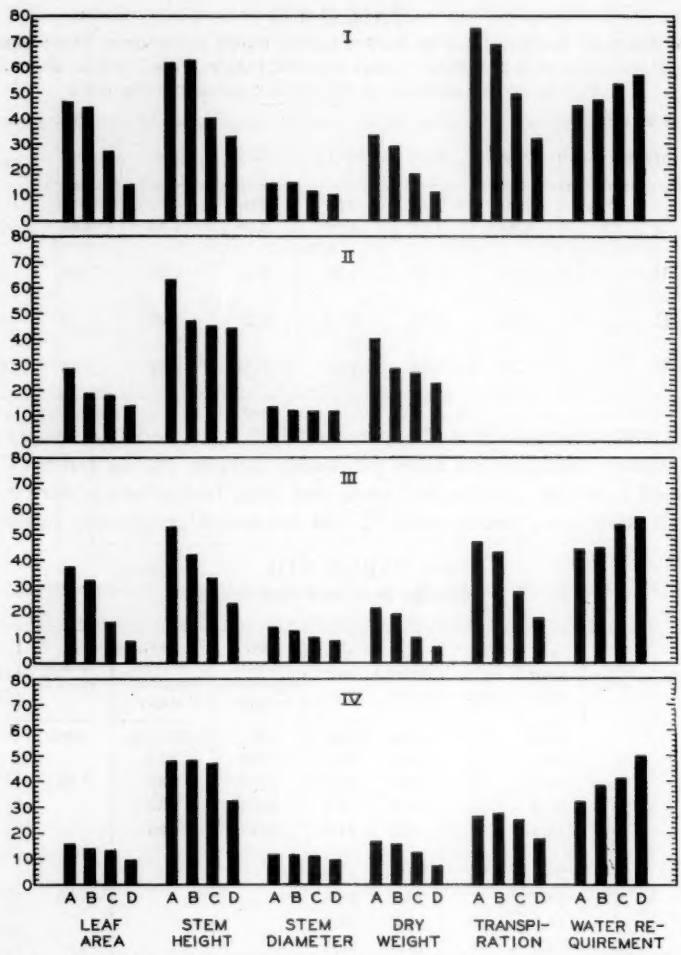

FIG. 8. Final plant measurements, total transpiration, and water requirement for all series. The ordinate represents leaf areas in square decimeters, stem heights in centimeters, stem diameters in millimeters, and dry weight in grams, but only 1/100 of the total transpiration in grams and 1/5 of the water requirement in grams of water per gram dry weight of material produced.

For the first week after subjection to wind, sets A and B had the same transpiration rate, while sets C and D were a little less. Thereafter, however, the transpiration rates per unit area increased with the wind, reaching a maximum for the highest velocities during the last two weeks of the series of from 35 to 50 per cent. more than the control.

The daily march of transpiration near the end of the series is represented by the data from series II given in table VII and shown graphically in figure 7. The transpiration rate at all times of day increased with wind velocity, but the influence is relatively greater at night than during the day. For example, set D (15 m.p.h. wind) at night used water from four to five times as rapidly as the control, while during the day only about 1.4 times. Measurements of stomatal openings during such series showed that for the greater part of the first two weeks the stomata were kept partially closed by the two highest winds, but thereafter wind had no noticeable effect on their diurnal cycle.

The final plant measurements from all four series are given in table VIII and are shown graphically in figure 8. In all series, leaf area, stem height and diameter, dry weight, and total transpiration (with one exception) decreased with increasing wind velocity. Inversely, the water requirement increased, the maximum increment observed being about 50 per cent. in the case of the 15 m.p.h. wind in series IV. The total transpiration and water requirement for series II could not be obtained on account of an unexpected rain that permitted unknown amounts of water to enter the cans.

All series were qualitatively alike in that wind always caused a reduction in growth, although quantitatively there were differences, the greatest being that the effect of wind in the fall seemed to be a little greater than in the spring. In the fall, the 15 m.p.h. wind caused a reduction in dry weight to about one third that of the control, but in the spring to only about one half. Some of the observed differences are doubtless due to statistical fluctuations caused by individual differences, but some are probably caused by diversity in

TABLE IX

WEEKLY AVERAGES OF AIR TEMPERATURE (T) IN DEGREES FAHRENHEIT, AND RELATIVE HUMIDITY (RH) IN PERCENTAGE FOR THE FOUR SERIES

SERIES I			SERIES II			SERIES III			SERIES IV		
WEEK ENDING	T	RH	WEEK ENDING	T	RH	WEEK ENDING	T	RH	WEEK ENDING	T	RH
	°F.	%		°F.	%		°F.	%		°F.	%
10/9/33	62	83	3/19/34	57	87	10/1/34	69	44	3/4/35	51	78
10/16	60	68	3/26	58	86	10/8	62	83	3/11	48	76
10/23	64	53	4/2	64	68	10/15	61	83	3/18	55	75
10/30	57	91	4/9	59	89	10/22	58	89	3/25	48	74
11/6	60	70	4/16	57	85	10/29	60	78	4/1	53	89
11/13	61	52	4/23	58	84				4/8	56	88
11/20	57	77	4/30	65	71				4/15	54	86
			5/7	64	69				4/22	57	87
									4/29	59	80

other environmental factors, thus altering the effect of wind. Weekly averages of air temperature and relative humidity during the four series are given in table IX.

The response of an average plant from each of the four sets in series III, arranged in order of increasing wind velocity, is shown by figure 9. Winds of 10 and 15 m.p.h. caused the well known gnarled and stunted appearance that is characteristic of trees growing in windy habitats. The leaves were rougher, fewer, more wrinkled, and somewhat less fully expanded than those of the control. The number of stomata per unit area rose with increasing wind velocity and the corresponding failure of the leaf to expand, a count for series IV yielding 188, 199, 260, and 304 per square millimeter for sets A, B, C, and D respectively. Microscopic sections of the leaves,

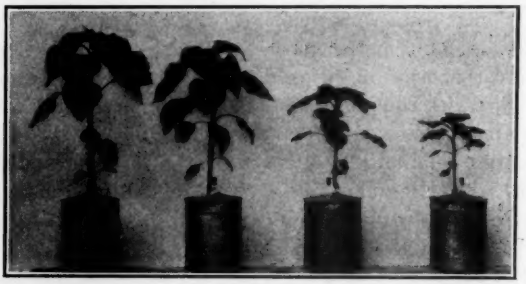

FIG. 9. Typical plants from the four sets in series III. Sets A, B, C, and D, with wind velocities of about 0, 5, 10, and 15 miles per hour respectively, are represented in order from left to right.

however, failed to show any material differences in the mesophyll, owing evidently to compensation by change of size. The stems, on the other hand, showed considerable divergence, particularly in the xylem elements. Camera lucida drawings of quadrants of the four stems, with only the xylem and bast strands outlined, are shown in figure 10. Stems of plants grown under the 15 m.p.h. wind (set D) showed 22 members of the first rank of bundles and only 6 of the second, while those of the control (set A) exhibited 22 of the first, 22 of the second, and 7 of the third. The intermediate sets fell between these two extremes. Although the number of members of the first rank of bundles was the same for both sets A and D, the number of vessels in each bundle was quite different. Each first rank bundle of A was made up of about 50 vessels while those of the same rank in D had only 30. Similarly, the area occupied by the xylem was considerably larger in A than in D, the former being 3.87 mm.^{2}, the latter 1.20. The cross-section area of the stems A and D were 113.0 and 36.6 mm.^{2}, respec-

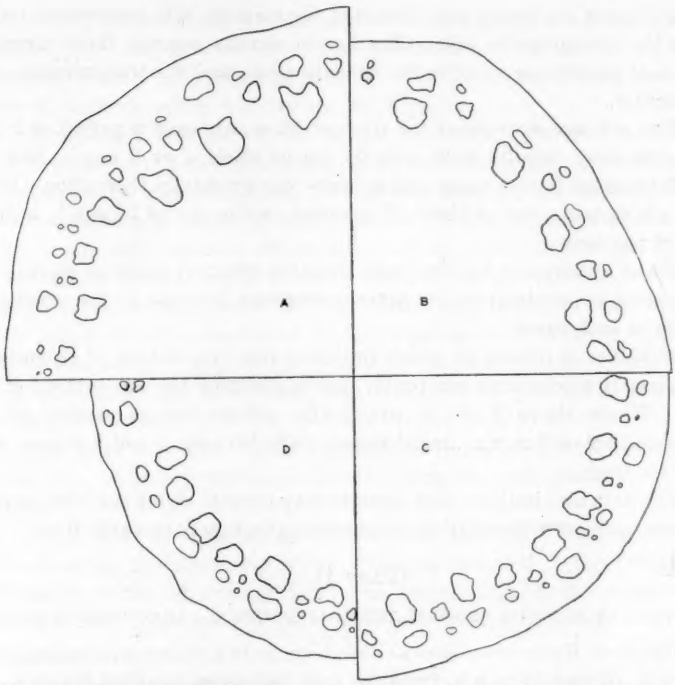

FIG. 10. Camera lucida drawings of quadrants of stems from typical plants from each of the four sets in series IV, with only the xylem and bast strands outlined.

tively; hence the proportion of the total stem area occupied by the xylem was practically the same in both cases, being 3.4 per cent. in the former and 3.3 in the latter.

Summary

PART I

EFFECT OF WIND ON TRANSPIRATION; SHORT TIME SERIES

1. The effect of wind on the transpiration rate of *Helianthus annuus* depends on the velocity. With velocities up to about 2 m.p.h., the transpiration rate increases at the onset of wind about 20 to 30 per cent. and maintains this value as long as the wind acts. For velocities above this, however, there is usually a rather high relative increase for the first few minutes after the wind is turned on, followed by a fall, which in turn is followed by a gradual increase. The initial increase in the rate of water loss rises with increasing velocity, reaching 138 per cent. in the case of the 16 m.p.h. wind. This causes

slight wilting of the leaves and closure of the stomata with consequent reduction in the transpiration rate. The leaves usually recover their turgidity within half an hour or so after the stomata close, and the transpiration rate rises slightly.

2. The average increase of the transpiration rate over a period of 2 to 4 hours rises very rapidly with velocity up to about 2 or 3 m.p.h., but the curve flattens out in this range and mounts only gradually thereafter. Wind of 1 m.p.h. causes a rise of about 30 per cent., while one of 16 m.p.h. induces about 50 per cent.

3. Wind apparently has the same relative effect at night as during the day, causing approximately the same percentage increase in the transpiration rate in both cases.

4. Evidence is presented which indicates that the closure of stomata by wind is partly mechanical and partly due to lowering the sap content of the leaves. Winds above 5 m.p.h. practically always induce closure of the stomata and below 2 m.p.h. almost never, while between 2 and 5 m.p.h., only part of the time.

5. The data also indicate that stomata may close to about one fifth of their maximum opening without affecting the transpiration rate markedly.

Part II

Effect of wind on growth and transpiration; long time series

1. Plants of *Helianthus annuus* have been grown under continuous winds of about 5, 10, and 15 m.p.h. from the first leaf stage to about 6 to 8 weeks of age, using large automobile fans driven by electric motors. Four series were made, two in the spring, two in the fall.

2. For the first week the transpiration rates of all three test sets were practically the same as that of the control, but thereafter the rate began to increase with increasing wind velocity so that by the end of the series the three test sets were using water at rates about 20, 35, and 50 per cent. higher per unit area of leaf surface than the control.

3. In all series, leaf area, stem height and diameter, dry weight, and total transpiration decreased with increasing wind velocity. The water requirement increased, the maximum increment observed being about 50 per cent. in the case of the 15 m.p.h. wind in series IV.

4. The depressing effect of wind on growth appears to be somewhat greater in the fall than in the spring. In the former cases, wind of about 15 m.p.h. caused a reduction in dry weight to about one third that of the control, but in the spring to only approximately one half.

5. In these series, the effect of wind on the transpiration rate is relatively considerably greater during the night than during the day. During

the day, the plants under the 15 m.p.h. wind transpired about 1.5 times as rapidly per unit area as the control plants, while during the night they transpired from 4 to 5 times as rapidly. However, the actual increase in grams of water is much less during the night.

6. Microscopic sections of the leaves of plants from the various sets failed to show any material differences in the mesophyll, due evidently to compensation by change of size. The stems, however, showed considerable difference, especially in the xylem elements. Stems of plants grown under the 15 m.p.h. wind showed 22 members of the first rank of bundles and only 6 of the second, while those of the control set exhibited 22 of the first, 22 of the second, and 7 of the third. Similarly, the area occupied by the xylem elements decreased with increasing wind velocity. However, the total cross-section area of the stems decreased in the same ratio so that the proportion of the total stem area occupied by the xylem showed no significant difference.

7. The plants grown under the higher wind velocities showed the gnarled and twisted appearance characteristic of trees growing in windy habitats.

8. Wind is to be regarded as a factor, though not the paramount one, in the dwarfing characteristic of the alpine tundra and in the reduced and procumbent forms of coastal dunes, as exemplified in the experimental areas on Pike's Peak and the dune gardens at Santa Barbara.

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FURTHER STUDIES OF ELONGATION AND EXPANSION IN *HELIANTHUS* PHYTOMETERS

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(WITH NINE FIGURES)

Introduction

The investigation of the factors and functions involved in elongation and expansion under reduced light intensity has been continued with the special purpose of relating these to structural modifications as well as to growth forms. The ultimate objectives in these studies are to measure in terms of adaptation the climates and edaphic habitats characteristic of the various transplant gardens and to permit interpreting the functional responses of natives not susceptible of phytometer treatment. At the same time, opportunity was taken to increase the number of light values to six, in order to simulate the whole range of natural habitats, as well as the control conditions in the lath-houses. In addition, an inquiry was made into the effect of size of container, and a battery of free phytometers was installed in the soil under each light intensity in order to integrate all the direct factors of the six eces.

Methods

The several light values were secured by means of lath-huts 6 ft. high and 8×12 in section. These were constructed of standard plaster lath $1\frac{1}{2} \times \frac{3}{8}$ inches in section, which were so spaced as to produce approximately the five intensities desired. The relative spacing and light values were as follows: (1) spacing $4\frac{3}{4}$ in., intensity 65 per cent. of full sunshine; (2) spacing $1\frac{3}{4}$ in., intensity 44 per cent.; (3) $\frac{3}{4}$ in. apart, 27 per cent.; (4) $\frac{3}{8}$ in. apart, 19 per cent.; (5) $\frac{3}{16}$ in. apart, 11 per cent. These values were approximately the same as in the large lath-houses used for producing adaptations, but the lowest of these had a rating of but 6 per cent., thus yielding six conditions beside full sunshine. Each hut was provided with a door of the same construction and after serious experience with on-shore winds, was firmly anchored at the four corners. Provision was also made for raising the huts several feet to accommodate height growth in the higher intensities. To facilitate weighing the exceptionally large number of phytometers, a hut 12 ft. tall and 6 ft. in cross section was built nearby and this served also for photographic purposes.

A stopwatch photometer was employed to measure intensities, and readings were taken hourly in the sun and in each hut for representative days during the growing season. For the exposure, the photometer was carried

through a wide circle with a uniform motion in order to integrate as accurately as possible the alternating bands of sun and shade. While this procedure does not insure exact results, it is the best so far devised for lathhouse conditions. The many exposures were then evaluated by the usual standard and graphs were made for the intensity through the day for the various habitats, using time of day for the abscissa and percentage of light as the ordinate. By using a planimeter to integrate under the curves, the the respective values were obtained in terms of full sunlight as 100 per cent. From the averages of these were obtained the final values for each hut, these being the mean of the total radiation for the day.

Four sizes of containers were employed, the largest being garbage cans 25 in. high and 17 in. in diameter; the others were respectively $11 \times 8\frac{1}{2}$ in., 10×8 in., and 7×6 in. These three were of the more recent type with conical lids and apertures 2 in. across to accommodate stem and watering tube. The cans were sealed with a mulch of fine dry sand and above this the opening was closed by a band of non-absorbent cotton wrapped about the plant stem. As a special provision against sudden rainstorms, rubber aprons were cut from old inner tubes in the form of circles 12 cm. in diameter, with a hole 1 cm. wide in the middle and a slit running to the margin. When the apron was placed around the stem, the edges of the slit were overlapped and held in place by paper-clips at the outer and inner margins. Contact between rubber and stem was prevented by a thin band of absorbent cotton. The conical shape of the apron led away the rain that might drip down the stem and at the same time prevented injury to the latter, in addition to promoting aeration in some measure. When rains promised to be heavy, canvas covers were placed above the batteries as an additional safeguard.

The soil employed was a good loam of the same quality as in previous experiments and similar technique was used in filling the containers uniformly, as well as in planting the seeds and caring for the seedlings (**3**). The four holards were set respectively at 35, 26, 18, and 14 per cent. of the dry weight of the soil, and six containers of each value were assigned to each of the six light intensities. Five seeds were planted in each container and allowed to germinate, but when the plumules were well developed, one seedling closest to the measured average for the series was selected and the others carefully removed. No further selection was made in organizing the batteries for the various conditions, the cans merely being grouped in accordance with the numerical sequence. All sets were kept in the greenhouse until the seedlings were well established; this period was two weeks for the fall and three weeks for the spring series. As a consequence, some slight differentiation due to holard was apparent at the time the batteries were distributed to the six light intensities.

At the outset, the phytometers were weighed at weekly intervals; but as they grew larger, weighing necessarily became more frequent, first daily and finally three times a day. The amount of water lost was replaced after each weighing in order to maintain the holard as nearly uniform as possible. For this purpose, a 500-cc. burette was employed, the water being delivered to the plant through a tube reaching into the lower part of the container. To avoid any possibility of harmful accumulation of salts from the hard water of the locality, rain water was utilized throughout the series. Measurements of stem height and diameter and of leaf dimensions were taken on the same day and in the same order each week, thus yielding a cumulative record of growth for the entire period. In spite of the fact that the 14 per cent. holard was but 2 to 3 per cent. above the echard for the soil and that the 35 per cent. was practically at the limit for adequate aeration, frequent watering and rate of loss so nearly balanced each other that the tendency toward wilting or an aeration deficit was rarely in evidence. The generally uniform distribution of the water in the soil is reflected in the root systems, which were well distributed and not compacted about the inner surface of the can (fig. 1).

Function and growth responses

Two seasonal installations were employed in this investigation. The fall-winter series was planted October 16, the phytometers distributed to the lath-huts October 30, and the experiment terminated February 11, 1935. The spring series was begun February 28; all cotyledons were expanded by March 8, at which time the various holards were standardized. These batteries were placed in the several situations on March 21 and the final results were secured on June 4. The period of growth for the first series was 119; for the second, 97 days. In both cases the plan had been to follow the course of development to flowering and fruiting, but a heavy gale flattened the lath-huts and abruptly terminated the fall series, destroying the leaves but injuring the heads only slightly. While the spring series suffered no such mishap, the weather conditions of a marine climate and the behavior of such a warm-temperate species as the sunflower precluded maintaining the series until all heads were mature.

The interval between the time of flowering in the sun and in the lowest light intensity was so long that plants in the higher light values lost one-third or more of their leaves. Thus, the average number of leaves produced in the sun and 35 per cent. holard was 37.7 per plant, while by the end an average of but 27 leaves remained, the lower 10 having dried and disintegrated. In 44 per cent. light, the average loss was less, *viz.*, 7 out of 39; in 19 per cent. 3 in 27; and in 11 per cent. 1 out of 22. This inevitably led to a cumulative discrepancy in the photosynthesis and transpiration of

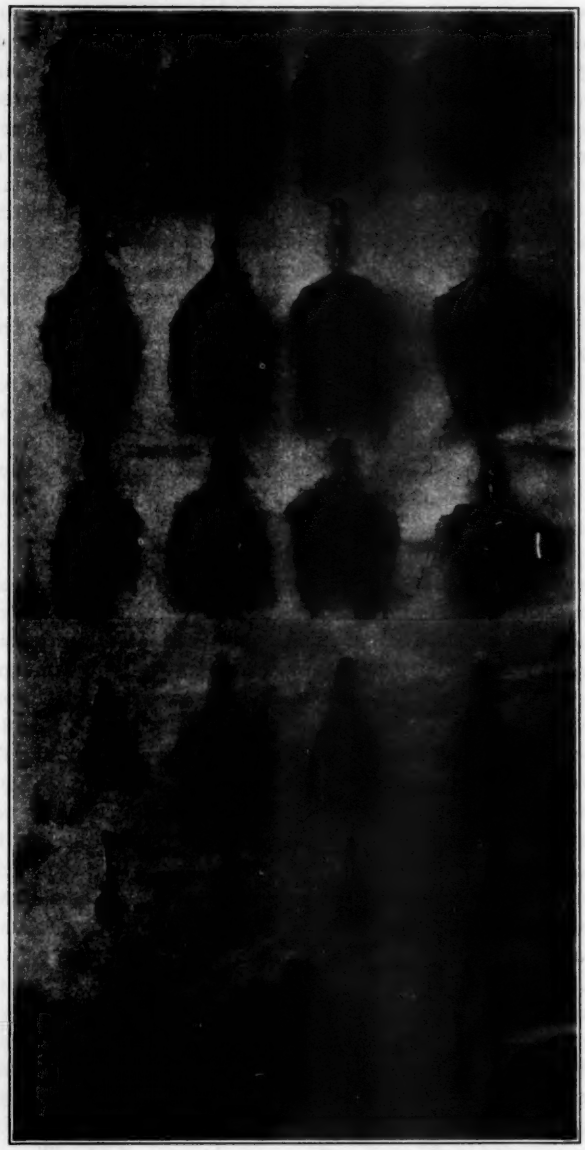


FIG. 1. Series of roots through the four holards and six light conditions.

TABLE I

AVERAGE OF MEASUREMENTS ON JUNE 4 FOR THE LARGE PHYTOMETERS

FACTOR GRADIENTS		STEM				H/D	LEAF AREA PER PLANT		LEAF NUMBER				TRANSPIRATION		WEIGHT OF PLANT			WATER REQUIREMENT	
LIGHT	HOLARD	HEIGHT		DIAMETER					TOTAL		JUNE 4		TOTAL GM. PER PLANT	TOTAL	WET	DRY	DRY		
%	%	*cm.*	%	*cm.*	%		*sq. cm.*	%		%		%		*gm./sq. cm.*	*gm.*	*gm.*	%	*cc./gm.*	%
100	35	138.5	100	2.30	100	60.4	5775.0	100.0	37.7	100	26.0	100	18533	3.21	627.1	127.7	100.0	145.3	100
	26	127.9	92	2.04	89	62.5	5180.3	89.8	36.0	95	25.3	97	15194	2.93	508.8	106.8	83.5	142.2	98
	18	111.1	80	1.74	76	63.9	4372.0	75.8	32.2	85	27.7	106	8674	1.83	383.0	67.9	53.1	127.7	88
	14	83.2	60	1.07	47	77.8	2574.0	44.6	26.3	70	22.3	86	4938	1.92	209.8	39.2	30.6	125.9	87
	Ave.	115.2	83	1.79	78	66.1	4475.0	77.6	33.0	88	25.3	97	11835	2.47	432.2	85.4	66.8	135.3	93
65	35	172.0	124	1.85	80	93.0	4626.0	80.1	38.0	101	28.5	110	18448	4.00	507.3	105.4	82.5	175.0	120
	26	135.0	97	1.73	75	78.0	3465.3	60.0	32.2	85	25.3	97	14525	4.20	380.5	79.8	62.4	182.0	125
	18	104.0	75	1.30	57	80.0	3560.3	61.6	29.0	77	22.7	87	9123	2.56	306.1	63.7	49.9	143.2	99
	14	85.2	62	1.10	48	77.5	3022.6	52.4	29.0	77	25.7	99	5322	1.77	230.7	39.9	31.2	133.4	92
	Ave.	124.0	90	1.49	65	82.1	3668.4	63.5	32.1	85	25.5	98	8223	3.14	356.2	72.2	56.5	158.4	109
44	35	191.0	138	2.13	93	89.6	7562.3	131.0	39.0	103	31.7	122	17562	2.32	577.6	105.5	82.5	166.4	115
	26	180.5	130	1.90	83	95.0	6884.0	119.2	31.7	84	25.5	98	13801	2.01	379.0	77.1	60.4	179.0	124
	18	137.0	99	1.40	61	97.9	5080.3	88.0	29.0	77	25.7	99	8529	1.68	308.6	59.1	46.3	144.3	99
	14	107.2	77	1.03	45	104.1	2984.3	51.6	29.3	78	26.0	100	4287	1.43	251.9	30.6	24.0	143.9	99
	Ave.	153.9	111	1.61	71	96.7	5627.7	97.4	32.3	86	27.2	107	11045	1.86	379.3	63.1	53.3	158.4	109

TABLE I.—(*Continued*)

Factor gradients		Stem				H/D	Leaf area per plant		Leaf number				Transpiration		Weight of plant			Water requirement	
									Total		June 4								
Light	Holard	Height		Diameter									Total gm. per plant	Total	Wet	Dry	Dry		
%	%	*cm.*	%	*cm.*	%		*sq. cm.*	%		%		%		*gm./sq. cm.*	*gm.*	*gm.*	%	*cc./gm.*	%
27	35	186.0	134	1.71	74	108.8	9645.3	167.0	35.0	93	32.0	123	16435	1.70	499.5	61.8	48.4	265.9	183
	26	192.0	139	1.53	67	125.3	6814.3	117.9	33.0	88	29.7	114	10515	1.55	358.4	57.6	45.1	182.8	126
	18	151.0	109	1.40	61	107.9	4676.3	81.0	30.3	80	28.0	108	6347	1.36	245.6	35.3	27.6	179.8	124
	14	101.7	73	1.03	45	98.6	2901.3	34.7	28.0	74	25.3	97	3672	1.83	143.2	20.6	16.1	178.2	123
	Ave.	157.7	114	1.42	62	110.1	5784.3	100.2	31.6	84	29.0	111	9242	1.61	311.7	43.8	34.3	201.7	139
19	35	200.0	144	1.56	58	128.0	5343.0	92.6	27.6	73	24.6	95	7512	1.41	295.0	24.9	19.5	301.5	208
	26	161.9	117	1.26	55	128.1	3613.1	62.6	27.0	72	25.7	99	4800	1.33	191.2	17.8	13.9	269.6	185
	18	141.0	102	1.00	44	141.0	2628.7	45.5	27.0	72	24.3	93	3538	1.35	133.3	15.9	12.4	222.5	153
	14	102.5	74	.83	36	123.3	1776.6	30.8	25.0	66	22.3	86	2108	1.19	83.5	11.2	8.8	188.2	130
	Ave.	151.3	109	1.16	48	130.1	3440.3	57.9	26.7	71	24.2	94	4490	1.32	175.7	17.5	13.7	245.4	169
11	35	139.0	100	1.13	49	123.0	2279.0	39.4	21.3	82	22.3	59	3018	1.33	96.3	7.5	5.9	402.5	277
	26	130.6	94	1.00	44	130.6	1491.6	25.8	19.6	75	22.3	59	1888	1.27	65.2	5.3	4.2	356.2	245
	18	113.5	82	0.83	36	136.8	1390.5	24.1	21.3	82	21.3	56	1637	1.18	55.8	5.3	4.2	308.8	212
	14	76.0	55	0.70	30	108.6	792.9	13.7	20.0	77	23.6	60	620	.78	37.4	2.9	2.3	213.8	147
	Ave.	114.7	83	0.91	40	124.7	1488.5	25.7	20.5	79	22.4	58	1791	1.14	63.7	5.3	4.1	320.3	220

the several light batteries, and necessitated closing the experiment before the buds opened in the weaker light intensities. In spite of its many advantages, which are distinctly greater than those of any other species so far utilized for standard phytometers, *Helianthus annuus* possesses the two defects of blocking off the lower leaves and of growing slowly in cold seasons such as that of 1934–1935 at Santa Barbara, as also in the climate of the alpine tundra on Pike's Peak.

The results for transpiration and water requirement, as well as for growth in terms of stem, leaf, and dry weight, were in close accord for the two series. The agreement between these and the five series discussed in the previous paper was likewise striking. Since in the present experiment there were six light intensities instead of four, the detailed results are given for light: holard in table I and these are summarized in the next paragraph. The weights were also determined separately for stem, leaves, roots, and heads, as shown in table II. In addition, a comparison is made between the results obtained from the four sizes of containers, not merely in connection with the two series, but also to throw light upon the phytometer method itself.

Under each light intensity, stem height was greatest in 35 per cent. holard and decreased consistently with the reduction to 14 per cent., with a single exception, the maximum of 200 cm. falling at 19 per cent. light and 35 per cent. water content (fig. 2). The greatest average height for the four holards occurred in 27 per cent. light, but 44 and 19 per cent. yielded values only a little less, while the shortest stems were found in sun at 115.2 cm. and 11 per cent. light at 114.7 cm. The diameter of the stem fell off steadily from 35 to 14 per cent. holard in every battery, ranging from the maximum of 2.3 cm. in the sun to 1.13 cm. in 11 per cent. light intensity. The average for each light value decreased from 1.79 cm. in the sun to 0.91 cm. in the deepest shade, that for 65 per cent. light being in disagreement here as in some other places, owing to leakage in a few containers during a heavy rain.

The total leaf area per plant diminished from 35 to 14 per cent. with great regularity, the one exception occurring at 65 per cent. light and 26 per cent. holard. However, the maximum was at 27/35 per cent. with a value of 9645.3 sq. cm. and the minimum of 792.9 at 11/14 per cent., a ratio of approximately 1:12. The highest average was found in 27 per cent. light, followed closely by 44 per cent.; the average area in the sun was three times greater than that for the lowest light intensity. The total number of leaves produced dropped regularly with the holard for the first four light intensities, but was almost constant for the two lower values, the range being from 39 to 21. The average number for each battery fell from 33 in the sun to 22 at 11 per cent. The number of leaves present at the close

TABLE II

WET AND DRY WEIGHTS OF PLANT PARTS FOR THE SPRING SERIES

Factor gradients		Wet weight					Dry weight				
Light	Holard	Stem	Leaves	Root	Flower	Total	Stem	Leaves	Root	Flower	Total
%	%	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
100	35	364.5	128.8	125.7	25.8	644.8	83.8	24.4	34.7	5.30	148.20
	26	313.0	145.8	125.7	14.0	598.5	73.7	26.1	19.4	2.73	121.90
	18	207.8	116.5	53.5	5.4	383.2	34.3	19.5	7.8	1.11	62.70
	14	113.0	69.1	32.0	14.2	228.3	18.4	11.6	4.0	2.62	36.60
	Ave.	249.6	115.1	84.2	14.8	463.7	52.5	20.4	16.5	2.94	92.30
65	35	337.8	88.6	70.0	8.8	505.2	74.5	19.4	21.6	2.13	117.63
	26	250.0	66.8	49.2	2.5	368.5	52.7	12.3	12.6	0.60	78.20
	18	100.7	46.8	44.5	70.0	262.0	24.7	10.2	7.7	14.96	57.50
	14	61.7	34.1	29.8	64.1	189.7	11.8	7.1	3.5	11.70	34.10
	Ave.	187.5	59.1	48.4	36.7	331.3	40.9	12.2	11.4	7.35	71.86
44	35	453.9	120.8	79.5	17.5	671.7	83.8	29.1	16.7	3.73	133.33
	26	284.1	124.5	41.2	28.1	477.5	50.9	22.3	9.7	5.70	88.60
	18	132.5	75.5	42.3	54.9	305.2	28.4	15.9	6.4	11.80	64.50
	14	89.7	43.5	25.8	25.7	184.7	15.0	10.8	2.1	5.00	32.90
	Ave.	240.1	91.1	47.2	31.6	409.7	44.5	19.5	9.0	5.43	79.83
27	35	342.3	121.2	51.5	1.2	516.2	36.9	18.1	9.5	0.44	64.94
	26	258.2	100.0	23.6	2.5	384.3	36.8	16.3	7.5	0.66	61.26
	18	154.2	58.5	11.0	8.1	231.8	20.0	10.7	3.1	1.75	35.50
	14	87.0	43.9	9.2	2.1	142.2	10.0	7.0	3.1	0.55	20.65
	Ave.	210.4	80.9	23.8	3.5	318.6	25.9	13.0	5.8	0.60	45.83

TABLE II.—(*Continued*)

Factor gradients		Wet weight					Dry weight				
Light	Holard	Stem	Leaves	Root	Flower	Total	Stem	Leaves	Root	Flower	Total
%	%	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
19	35	206.0	50.7	8.3	.0	265.0	16.7	8.5	1.5	0.13	26.83
	26	125.0	28.0	8.0	.0	161.0	11.0	4.4	1.5	0.30	17.20
	18	84.8	18.6	4.3	.0	107.7	8.6	3.2	1.0	0.30	13.10
	14	48.8	19.5	4.4	.6	73.3	5.8	3.3	1.4	0.22	10.72
	Ave.	116.2	29.2	6.3	.6	151.9	8.4	4.9	1.4	0.24	16.96
11	35	79.7	20.0	3.7	0	103.4	4.6	2.6	0.72	0	7.92
	26	50.0	11.0	1.7	0	62.7	3.1	1.9	0.32	0	5.32
	18	38.2	10.2	2.0	0	50.4	2.0	2.4	0.52	0.04	4.95
	14	16.5	6.3	1.0	0	23.8	1.3	1.1	0.30	0.03	3.03
	Ave.	46.1	11.9	2.1	0	60.1	2.7	2.0	0.46	0.04	5.31

was greatest in the intermediate light values, namely, 29 at 27 per cent. and 27 at 44 per cent., and least in 11 per cent. However, the highest average loss of leaves was 8 in the sun, falling to 7, 5, 2.5, and 2 at 11 per cent.

With respect to total transpiration per plant, the descending order was entirely consistent with the reduction in holard. In the first three light

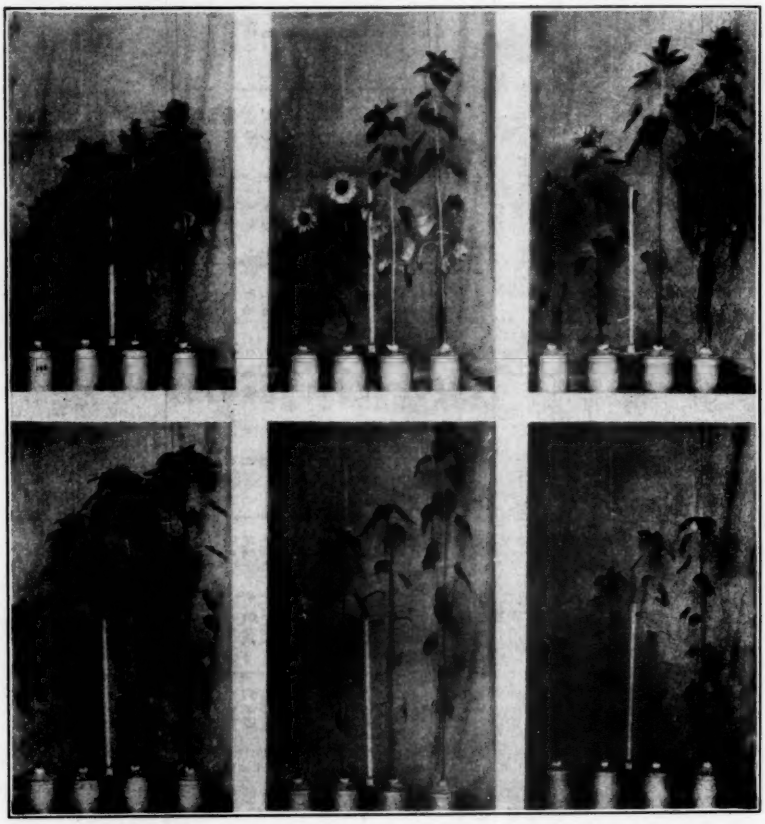


FIG. 2. Representative phytometer sets from each light habitat, arranged according to holards 14, 18, 26, and 35 per cent.

values the loss in 35 per cent. holard was nearly four times as great as in 14 per cent., and in 11 per cent. light, five times as much. The average loss for each light battery was in accord with diminished intensity, except for 65 per cent., as just explained. The loss in grams per sq. cm. decreased with the holard with fair regularity, while it agreed with the light intensity,

with the exception of 65 per cent. again. As a rule the water requirement was highest in the holard of 35 per cent., with two slight exceptions for 26 per cent., and uniformly lowest in 14 per cent. The average water requirement rose consistently with reduced light from 135.3 cc./gm. for sun to 320.3 cc./gm. for the lowest light value, apart from the same figure for 65 and 44 per cent.

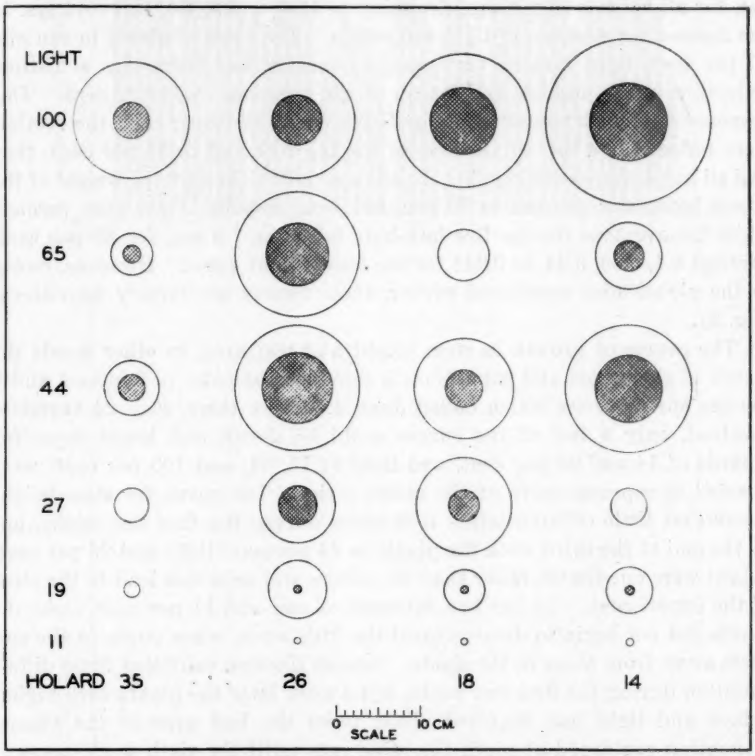


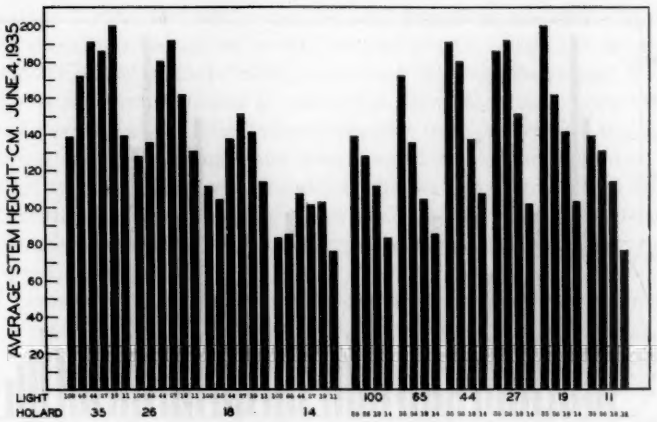
FIG. 3. Comparative diagram of flower heads; disk cross-hatched, rays plain, buds clear.

Wet weight of plant exhibited a complete correspondence with holard, falling off with much uniformity from 35 to 14 per cent., while the dry weight was equally consistent. This was likewise true for the averages, the respective values for the six light intensities from sun being 85.4, 72.2, 63.1, 43.8, 17.5, and 5.3 gm. When the organs of the plant were weighed separately, the wet weight of the stem decreased consistently, as did that of leaves and roots with but one or two slight discrepancies. The dry weights of all three gave similarly accordant results, the maximum of 83.8 gm. for

the stem falling in 100/35 per cent. and the minimum, 1.3 gm., in 11/14 per cent., the averages for the light extremes being 52.5 and 2.7 gm. The maximum for the leaves was 29.1 and the minimum 1.1 gm., with the average for the sun 20.4 gm. and for 11 per cent. light, 2 gm. The highest value for roots fell at 35 per cent. holard in sun, namely, 34.7 gm., while the lowest, 0.3 gm., was found at 11/14 per cent. The average weight was 16.5 gm. for all holards in the sun, dropping to 11.4, 9, 5.8, 1.4, and 0.46 gm. in the descending sequence of light intensities. The roots of plants in sun and 65 per cent. light formed very compact masses, but there was a distinct reduction in the number and length of the branches in 44 per cent. This response was much more marked in 27 per cent.; in 19 per cent. the rootlets were reduced to a tuft at the base of the tap root and in 11 per cent. they had all but disappeared (fig. 1). In all cases but in the sun, the weight of the flower heads was greatest in 18 per cent. holard, with 14 per cent. second; while the averages for the five lath-huts fell from 7.3 gm. for 65 per cent. through 5.4, 0.59, 0.24, to 0.018 for the lowest light value. However, owing to the vicissitudes mentioned earlier, these figures are largely provisional (fig. 3).

The course of growth in stem height and leaf area, in other words the march of elongation and expansion, is shown graphically in figures 4 and 5 for the spring series which ended June 4. Since there were 24 variables involved, only a few of the curves could be shown and hence those for holards of 14 and 26 per cent. and light of 11, 44, and 100 per cent. were selected as representative of the entire series. The curve for stem height shows that little differentiation took place during the first two weeks, but by the end of the third week the plants in 44 per cent. light and 26 per cent. holard were one-fourth taller than the others and held this lead to the close of the experiment. At the two extremes of sun and 11 per cent. light the plants did not begin to diverge until the fifth week, when stems in the sun drew away from those in the shade. Leaves likewise exhibited little differentiation during the first two weeks, but a week later the plants with higher holard and light had acquired about twice the leaf area of the others. Expansion continued at much the same rate until the sixth week, when it became definitely greater for the 44 per cent. light. The lowest holard counterbalanced higher light values and the individuals in sun and 44 per cent. light did not diverge until the ninth week.

The influence of size of container was made the subject of a special inquiry, which took account of free phytometers as well as the four types of can indicated earlier. The standard container when complete weighed 15 kg., the intermediate 12 kg., and the smallest 4.5 kg., while the garbage cans reached a total of 110 kg. when filled. The latter were employed as controls, chiefly for the batteries of free phytometers which could not be



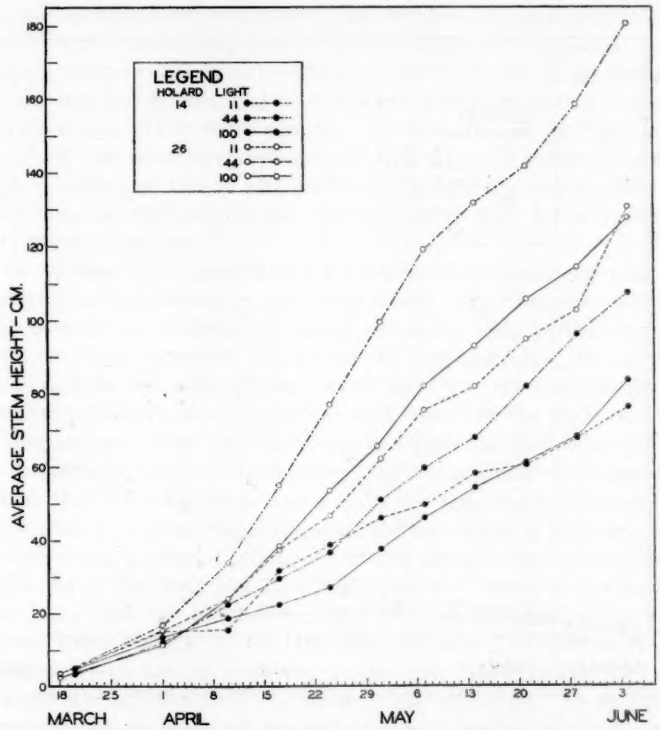
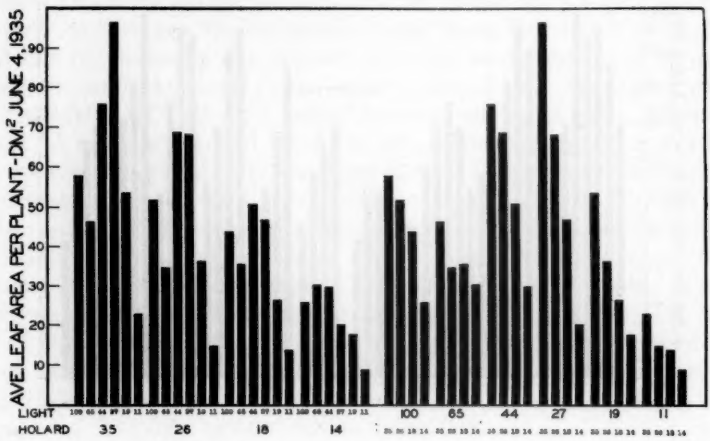


FIG. 4. March of average stem height for series 2, and final values on June 4.



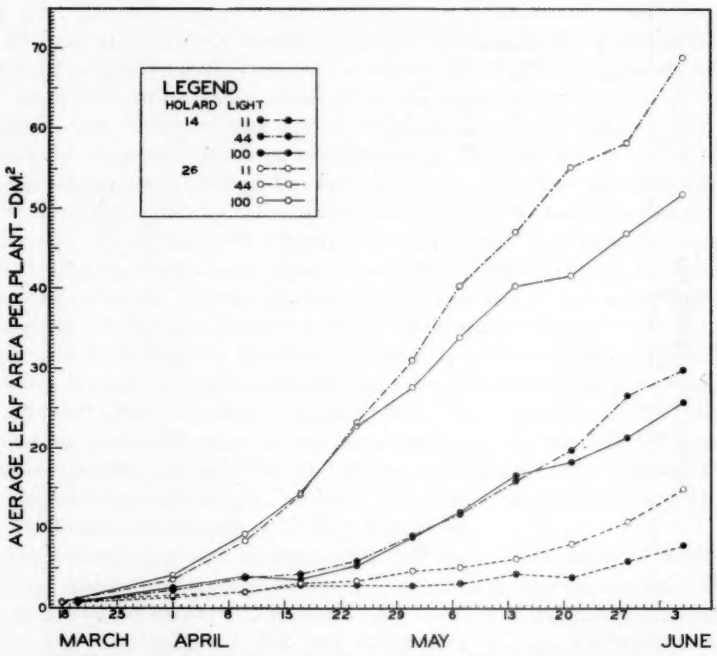


FIG. 5. Increase in leaf area during series 2, and final values on June 4.

weighed, and were but six in number, all placed in the sun. The three sizes of containers amounted to 348 in number, of which 148 belonged to the large phytometer, there being 24 in each light intensity and 6 in each holard. A measured amount of water was given each day, after they had attained a certain size, to the free phytometers in the soil under the six light conditions, and the garbage cans were treated in the same manner. This amounted to giving both sets practically all the water they could use; this fact probably has some bearing upon their slower growth at first when aeration may well have been a factor, as well as upon their much more rapid growth later.

As a consequence, no strict comparison can be made between the growth of these plants and those in the standard containers. They do confirm the experience derived from many phytometer series through two decades to the effect that number and size of container must always be balanced against each other. With 24 sets of conditions, as in the 9 series that have dealt with elongation and expansion, phytometers of 15 kilos. constitute the practicable maximum for a number of 200 or more that will require weighing two to three times daily before the experiment is completed. On the other hand, such a capacity sets a definite limit to growth in the better conditions of light and holard, and phytometers of this size have a dependable life period of but two to three months. By contrast, the garbage-can type of the larger sizes permits carrying such large plants as sunflower and corn through to maturity, but as phytometers to measure a considerable range of conditions, especially in nature, they demand a staff and equipment all but impossible to realize.

A comparison of the growth in the three sizes of phytometer cans at the end of the first three weeks gave a stem height respectively 35 and 40 per cent. greater in the medium and large containers than in the small ones. Likewise, the stem diameter was 21 and 35 per cent. and the number of leaves 40 and 50 per cent. greater, while the leaf area was 133 per cent. more for the medium cans and 52 per cent. more for the large ones. The small phytometers were dismantled at this time as they were evidently reaching the limit of container capacity, and the medium ones were discontinued on May 1 for the same reason. At this time the individuals of the large set were 11 per cent. taller and of the free series 9 per cent. shorter than those in the medium containers and the garbage cans, but in contrast the diameter of the stem was 19 and 14 per cent. more in the large and garbage cans than for the medium and free phytometers. The average number of leaves was 21 for the three sets, but only 14.6 for the free phytometers, in which the leaf area was 24 per cent. less than for the medium cans, while it was 2–4 per cent. greater in the other two. From this time the plants in the garbage cans began to forge ahead and the free phytom-

eters to grow more rapidly than those in the large cans. At the end of the experiment on June 4, the relative values were as follows for the large, free, and garbage-can phytometers: stem height 100, 116, 136; stem diameter 100, 131, 167; number of leaves 100, 128, 149; leaf area 100, 237, 429.

The remaining three sets were taken off to permit determining wet and dry weights at the end of 12 weeks, with the results shown in table III.

TABLE III

WET AND DRY WEIGHTS OF THE THREE FINAL SETS

	PERCENTAGE LIGHT					
	100	65	44	27	19	11
	WET WEIGHT					
	gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Large cans	627.1	507.3	577.6	499.5	295.0	96.3
Free phytometers	1394.9	1265.5	1239.6	323.4	142.6	29.6
Garbage cans	2340.8					
	DRY WEIGHT					
Large cans	127.7	105.4	105.5	61.8	24.9	7.5
Free phytometers	204.8	168.7	127.9	43.7	13.8	2.0
Garbage cans	438.3					

For both wet and dry weights, the values for the garbage-can controls were approximately twice as high as for the free phytometers, and for these about twice as great as for the large phytometers, in the case of sun conditions. Both of the latter fall off consistently in weight with decreasing light intensity, and most rapidly in the lower intensities. The large cans begin to exceed the free plants at 27 per cent. light and both wet and dry weights are more than three times greater in 11 per cent. The cause of this reversal in behavior requires further investigation, as does also the striking difference in the three sets in the sun. This bespeaks better conditions in the larger soil masses, partly to be explained by the generous watering, and the drop in the free phytometers is most plausibly explained by the effect of this upon air content in the three lower light values.

Structural modifications

In addition to the striking responses in growth and form of stem, leaf, and roots, corresponding or compensating adaptations occurred in the intimate structure of these organs. These were especially exhibited by the stomata, as seen in tables IV and V.

The epidermis was stripped from the leaf and immediately placed in absolute alcohol in accordance with the usual technique, a median leaf being selected in each of the 24 sets. The strips were taken from the proximate

TABLE IV

NUMBER OF STOMATA PER SQ. MM. IN UPPER AND LOWER EPIDERMIS THROUGHOUT THE LIGHT AND HOLARD SERIES

LIGHT	HOLARD 35 PER CENT.		HOLARD 26 PER CENT.		HOLARD 18 PER CENT.		HOLARD 14 PER CENT.		AVERAGE NUMBER	
	LOWER	UPPER	LOWER	UPPER	LOWER	UPPER	LOWER	UPPER	LOWER	UPPER
%										
100	315	288	341	280	258	240	248	240	290.8	262.1
65	293	286	221	221	228	218	203	195	236.2	230.1
44	165	174	180	184	195	195	180	190	180.1	185.9
27	120	129	145	146	142	147	135	140	135.5	140.5
19	105	120	116	125	165	165	131	135	129.4	136.4
11	90	116	90	117	95	112	123	138	99.6	120.8
Ave.	182	186	182	179	181	180	170	173	178.6	179.3

center of the leaf, about halfway from the base to tip and from midrib to margin. The major correlation in number of stomata is with light intensity, the decrease from 100 to 11 per cent. being all but entirely consistent for both lower and upper surfaces in each holard and perfectly so for the averages of each light value (fig. 6). These range from 291 in 100 per cent. to 99 in 11 per cent. for the lower epidermis and 262 and 120 for the upper. The averages for the four holards are not significant except in the 14 per cent., since averaging obscures the most novel point in the behavior as to number. This is the fact that stomata are most numerous in the lower epidermis for 100 and 65 per cent., but tend to become slightly more abundant in the upper for the next two intensities and definitely so in the two

TABLE V

SIZE OF STOMATA IN MICRONS FOR THE LOWER EPIDERMIS OF PLANTS THROUGHOUT THE SERIES

LIGHT	HOLARD 35 PER CENT.	HOLARD 26 PER CENT.	HOLARD 18 PER CENT.	HOLARD 14 PER CENT.	AVERAGE SIZE
%	μ	μ	μ	μ	μ
100	34.4 × 24.1	20.1 × 24.8	31.8 × 23.7	28.3 × 21.2	28.7 × 23.5
65	24.7 × 24.1	32.9 × 23.4	23.8 × 21.2	28.3 × 21.2	27.4 × 22.5
44	31.8 × 21.2	34.3 × 22.3	28.3 × 21.2	26.6 × 21.2	30.6 × 21.5
27	33.3 × 26.6	33.6 × 21.2	30.4 × 23.4	28.3 × 21.2	31.4 × 23.1
19	33.6 × 24.8	31.8 × 24.8	31.2 × 21.6	30.1 × 21.2	31.7 × 23.1
11	34.0 × 24.4	31.8 × 24.8	29.4 × 23.0	28.3 × 21.2	30.9 × 23.4
Ave.	32.0 × 24.2	31.6 × 23.6	29.1 × 23.0	28.3 × 21.2	30.0 × 22.8

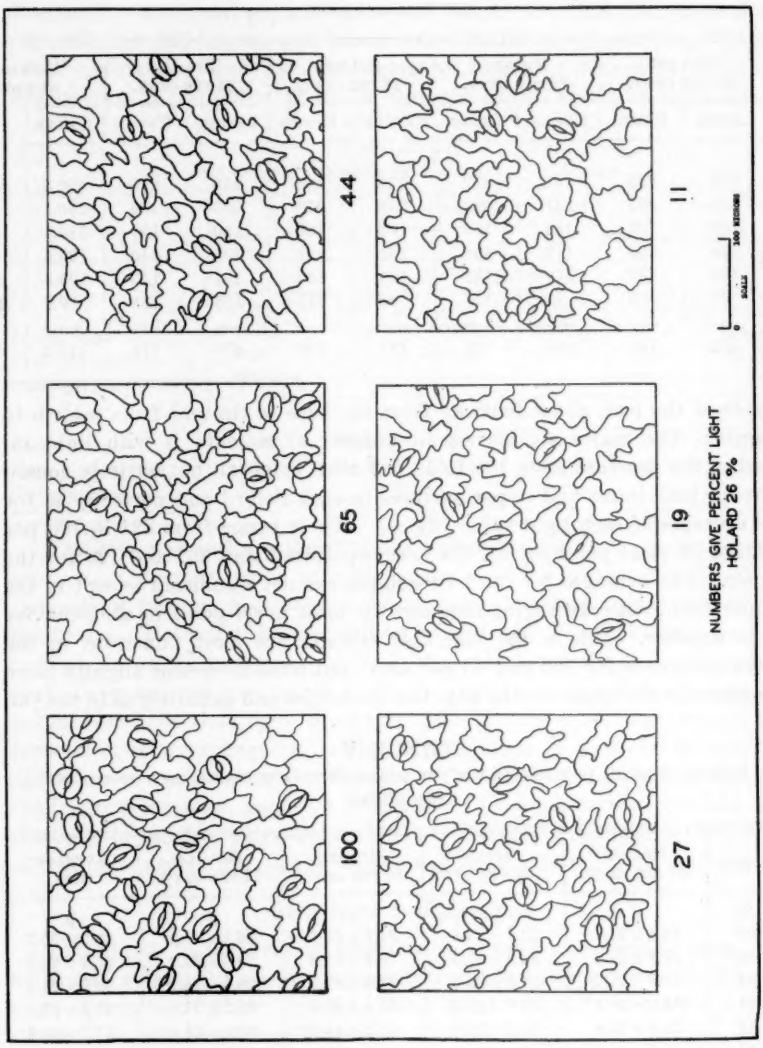


FIG. 6. Modification of stomatal number in the six light intensities.

lower values. While the numbers generally follow the relative expansion of the leaf, the change in preponderance from lower to upper surface makes it clear that the number of stomatal initials varies under the several conditions, not merely between surfaces but also between leaves and to a considerable extent independent of the degree of expansion.

Modification in size bears no evident relation to light intensity in any particular holard, but the average size for the three higher intensities is distinctly less than for the three lower ones. The chief difference, however, is to be found in the averages for the respective holards, the largest size occurring in 35 per cent. and this diminishing regularly to 14 per cent.

Quantitative changes in the mesophyll are reflected in the thickness of leaf, while the qualitative ones naturally affect the mesophyll, either as direct responses to light and water or indirectly in relation to stomata and air passages. As may be seen in table VI, the thickness of the leaf was

TABLE VI

THICKNESS OF LEAF, PALISADE AND SPONGE TISSUES

Factor gradients		Leaf	Palisade	Sponge
Light	Holard			
%	%	μ	μ	μ
Large cans				
100	35	267	153	114
	14	192	111	81
65	35	248	142	106
	14	177	106	71
11	35	142	96	46
	14	142	78	64
Free phytometers				
100	35	238	131	107
65	35	230	126	104
44	35	202	128	74
27	35	156	92	64
19	35	149	78	71
11	35	149	89	60
Garbage cans				
100	35	258	135	124

greatest for the large cans in sun and 35 per cent. holard, followed in order by garbage cans, large can in 65/35 per cent., and free phytometers in sun and 35 per cent. The general agreement between the large and free phytometers was close, the two falling off consistently and in much the same

degree from a maximum in sun to a minimum in 11 per cent. light. For the large plants, 35 per cent. holard produced a leaf one-third thicker than 14 per cent., except for the lowest light intensity, where the values were the same. In this set the values for palisade and for sponge decreased

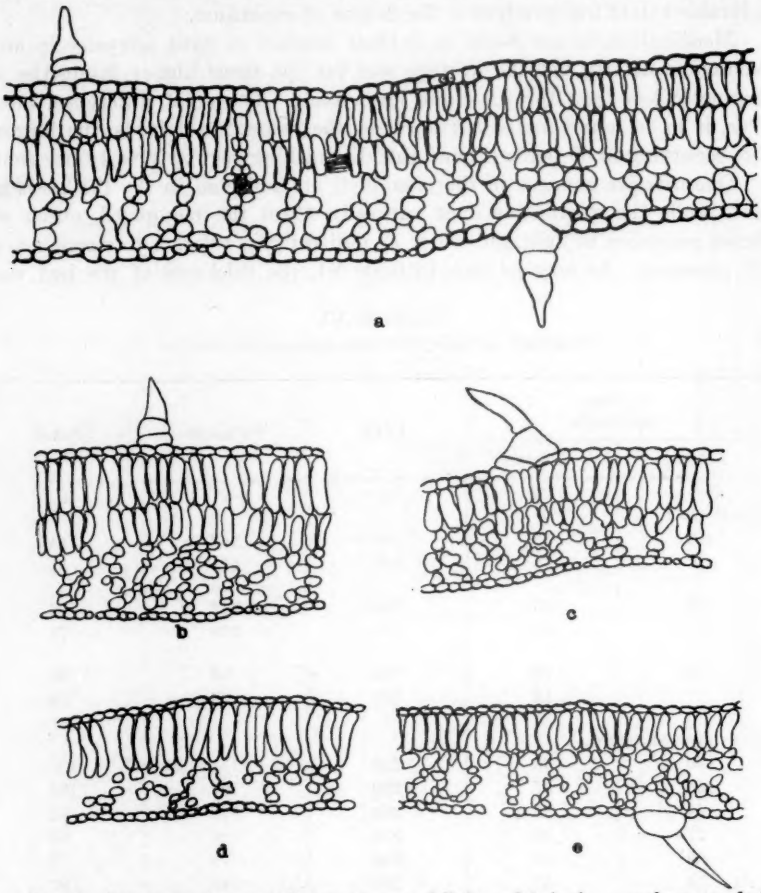


FIG. 7. Structure of mesophyll in extremes of light and holard: *a*, garbage-can battery; *b*, *c*, 65 per cent. light, 35 and 14 per cent. holard; *d*, *e*, 14 per cent. light, 35 and 14 per cent. holard. ×150.

regularly with reduced light, except for 11 per cent., and with this same discrepancy, each tissue was thicker in 35 per cent. than in 14 per cent., in accord with the behavior of the leaf itself. The free phytometers were somewhat less harmonious, but there was general reduction from sun to 11 per cent. light.

The mesophyll likewise exhibited structural modifications in close correlation with the factor dosage, as illustrated in figure 7. With the cross section of a median leaf from a garbage-can phytometer as the standard, a large-phytometer leaf from 65/35 per cent. agrees in having two full rows of palisade cells and essentially the same type of sponge tissue. However,

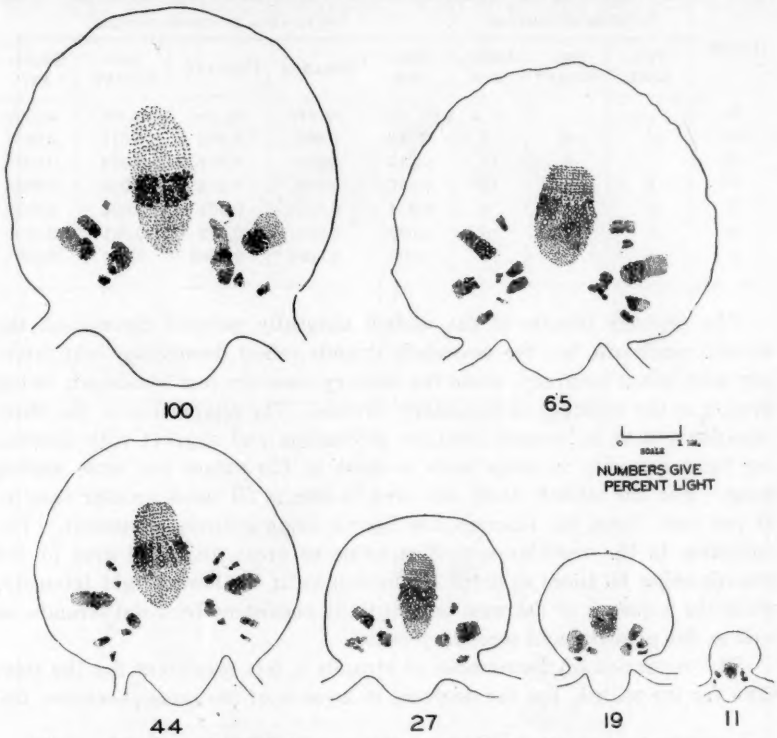


FIG. 8. Diagram of midrib in cross section, showing modification of fibrovascular system; 26 per cent. holard.

leaves in the same light intensity but in 14 per cent. holard had reduced the inner palisade cells to about half the length and the sponge also in some measure. In 11/35 per cent., the inner row of palisade had completely disappeared and the sponge tissue was much looser in texture, while 14 per cent. holard in the same light produced a shorter outer row of palisade, with the inner reduced to globoid cells.

Cross sections of representative portions of the midrib were employed for comparison of the modification of the conductive-supportive tissues. These differences are best appreciated in the graphic form (fig. 8), but

an effort has been made to express them in quantities also, as shown in table VII.

TABLE VII

MODIFICATION OF THE FIBROVASCULAR SYSTEM IN MIDRIB; 26 PER CENT. HOLARD

LIGHT	NUMBER OF STRANDS			TOTAL AREA IN CROSS SECTION				
	PRIMARY	SECONDARY	TERTIARY	MIDRIB	STRANDS	PRIMARY	SECONDARY	TERTIARY
%				*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*	*sq. cm.*
100	1	4	6	0.268	0.036	0.022	0.011	0.0021
65	1	3	11	0.218	0.023	0.014	0.004	0.0055
44	1	2	13	0.147	0.022	0.014	0.003	0.0054
27	1	2	6	0.074	0.011	0.007	0.002	0.0023
19	1	1	6	0.039	0.005	0.002	0.001	0.0012
11	1	0	4	0.008	0.0006	0.0005	0.000	0.0001

The primary bundle of the midrib naturally persists throughout the several conditions, but the secondary strands reflect decreasing light intensity with much accuracy, while the tertiary ones are less consistent, owing in part to the splitting of secondary strands. The adaptation of the fibrovascular system to lessened need for conduction and support with decreasing light intensity is much more evident in the values for cross section areas. For the midrib itself, the area in sun is 30 times greater than in 11 per cent. light, the intermediate figures being entirely consistent. The reduction in the vascular system is twice as great, the total area of the strands being 60 times as much in the sun as in the lowest light intensity, while the sequence of decrease is essentially consistent for total strands, as well as for primary and secondary ones.

The reduction of the number of strands is less consistent for the stem than for the midrib, but the decrease in areas is of the same character, fol-

TABLE VIII

MODIFICATION OF FIBROVASCULAR SYSTEM OF STEM; 26 PER CENT. HOLARD

LIGHT	NUMBER OF STRANDS		TOTAL AREA IN CROSS SECTION	
	PRIMARY	SECONDARY	STEM	STRANDS
%			*sq. cm.*	*sq. cm.*
100	12	8	0.95	0.198
65	10	6	1.18	0.200
44	10	6	0.88	0.117
27	11	9	0.43	0.062
19	6	6	0.27	0.018
11	8	6	0.1	0.011

lowing the light intensity closely, with the exception of a small discrepancy in 65 per cent. (fig. 9). Moreover, while the stem area at 11 per cent. is seven times smaller than for the sun, that of the strands is 18 times less, or more than twice as much. In general the stem appears to be somewhat more plastic than the midrib, and this assumption is supported by the fact that under the pressure of continuous wind of varying velocities, bundles

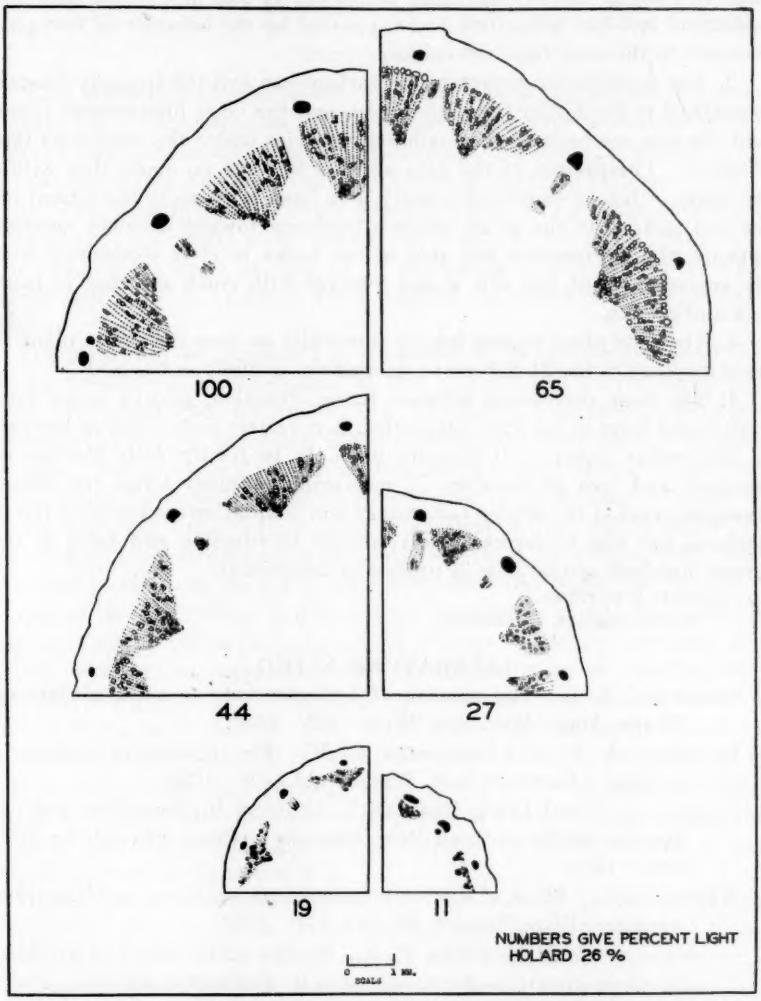


FIG. 9. Diagram of stem quadrants in cross section showing modification of fibrovascular system.

increase in number relatively more rapidly than in size and the bast strands receive special emphasis.

Summary

1. The conclusions reached heretofore as to the functional and growth responses of *Helianthus annuus* to combinations of light and holard and the relative rôles of the two are fully borne out in two new series with two additional lath-hut intensities, and supported by the behavior of free phytometers in the same light intensities.

2. The much better growth in the garbage-can and the free phytometers is confined to the higher light intensities, and the large phytometers represent the best compromise for sunflower batteries under the conditions that obtained. Comparison of the data appears to leave no doubt that within the range of holard employed, namely, 1 to 2 per cent. above the echard for the soil and up to the point where a tendency toward deficient aeration appears, the phytometers not only utilize water in close correlation with the amount present, but this is also reflected with much accuracy in function and growth.

3. The four plant organs behave essentially as does the entire plant in their response to the 24 different combinations of light and holard.

4. The basic correlation between factor, function, growth as an integrator, and form as the final adaptation, is regularly a close one in the case of *Helianthus annuus*. It appears not only to justify fully the use of standard and free phytometers in measuring in plant terms the varied transplant eces at the Alpine Laboratory and adaptation sequences at Santa Barbara, but also to forecast the responses in function and form of the several hundred species now in process of adaptation.

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OSMOTIC PRESSURE AND WATER CONTENT OF PRAIRIE PLANTS[1]

LAURENCE A. STODDART

(WITH FIVE FIGURES)

Introduction

Many conflicting ideas have been expressed concerning osmotic pressure in plant cells. The purposes of this investigation were to determine the range of osmotic pressure in prairie plants and to ascertain, if possible, any correlation between osmotic pressure and certain ecological factors.

Since the osmotic pressure of a solution is dependent upon the proportion of water and osmotically active substances, it may be concluded that either a decrease in the water content or an increase in osmotically active substances will have an identical effect, namely, an increase in the osmotic pressure.

The plant may be considered as a product of its environment, and the osmotic pressure as an expression of that environment's effect upon the plant. The plant lives in a dual habitat, and acts as a balancing factor between the moister soil surrounding the roots and the drier air surrounding the tops. The plant, as a connecting link between these habitats, expresses their relative forces by its osmotic value because the percentage of water in the plant depends upon the comparative demands of these two habitats. If the soil supplies moisture as fast as the air removes it, then the osmotic pressure must be low. As the air removes more and more water and the water content of the plant tissue is drawn upon in order to supply the demand, then, so long as the osmotically active substances remain constant, the osmotic pressure rises proportionately. Soil moisture and air humidity may therefore be regarded as the most important external factors of the environment, and the water content of the plant tissues may be considered as an extremely important internal factor in the study of osmotic pressure. It is believed that changes in osmotic pressure should be looked upon primarily not as an adaptation to the environment but as a result of environmental changes; thus high pressures in time of drought are chiefly a result of the drought and not an adaptation to it.

During the extremely dry summer of 1934 osmotic variations due to changes of water content far overshadowed those due to changes of sugar content, therefore the latter factor was not considered in any detail.

[1] Contribution from the Department of Botany, University of Nebraska, no. 95.

Literature

One of the most complete summaries of recent investigations on osmotic pressure is that given by MILLER (17). KORSTIAN (11) and MEYER (16) give good accounts of the methods of collecting material. For information on killing of material and extraction of sap, the papers of DIXON and ATKINS (1), GORTNER, LAWRENCE, and HARRIS (4), and MEYER (16) have been found helpful. Osmotic pressure as an ecological or environmental indicator is well treated by DRABBLE and DRABBLE (2), MCCOOL and MILLAR (14), KORSTIAN (11), MEYER (15), HARRIS, LAWRENCE, and GORTNER (8), MOLZ (18), and EATON (3). The indicator value of osmotic pressures is discussed by HARRIS *et al.* (7) and by WALTER (20). The daily and seasonal cycle was studied by HERRICK (10), MOLZ (18), MCCOOL and MILLAR (14), HENRICI (9), and KORSTIAN (11). HENRICI worked on the osmotic pressure of grasses (9). The relationship of osmotic pressure to drought and frost resistance is reviewed by MAXIMOV (13). Water content of tissues was studied by YAPP and MASON (24) and by MCCOOL and MILLAR (14). WALTER (20) alone has touched upon the osmotic pressures of prairie plants. He made determinations on a dozen species in the early spring of 1930 and again after a moderate midsummer drought.

Methods

All osmotic pressure determinations were made by the cryoscopic method. Material was gathered in as nearly as possible the same place throughout the season, in order to avoid soil moisture heterogeneity. Collections were made on either the lowland or upland prairie at Belmont, 2 miles north of Lincoln, Nebraska, between the hours of 1 and 3 P.M., unless otherwise noted. The plants were cut at ground level and placed immediately in pyrex glass beakers, corked, and frozen in carbon dioxide ice, where they were left overnight. The juice was extracted from the thawed material the following morning at a pressure of 10,000 pounds. The freezing point of the sap was determined at once by use of an ether evaporation device (fig. 1) much as described by MATHEWS (12). The thermometer measured to 0.001°, and, by means of close fitting tubes, a test could be made with only 2 to 3 cc. of sap. Atmospheric pressure was calculated according to the customary formula and tables by HARRIS and GORTNER (5, 6).

Water content was determined on samples of about 50 gm. collected in screw-top aluminum cans. The samples were dried at 80° C. for at least 48 hours. The calculations were made on the basis of green weight, to conform with other work on water content of plant tissues.

Soil moisture determinations were made at each collection and were designed to show the trend of available moisture rather than the specific

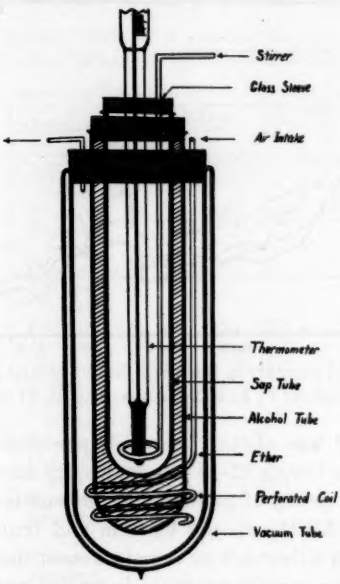

FIG. 1. Ether evaporation freezer used in cryoscopic measurements. Air is sucked from the lefthand tube by means of a water suction pump which causes air to enter at the righthand (intake) tube. The glass stirrer is operated constantly. About 10 minutes is ample time to freeze a sample.

moisture available to any one individual. Samples were taken at depths at 0–6 inches, 6–12 inches, 1–2 feet, and 2–3 feet. The Briggs' geotome and the customary friction-top soil cans were used. Calculations were made on the oven-dry basis.

Procedure

SEASONAL TREND

The summer of 1934 was the hottest and driest on record in Nebraska, and consequently the absolute extremes of values were doubtless met. The seasonal trend of the most important species shows a wide range both in osmotic values and in water content of the tissues. The values for shallowly rooted plants especially followed closely the graph of available water in the soil (fig. 2). The available water decreased throughout most of the season except for a period in early June when slight increases were recorded. The soil moisture in all levels down to 3 feet reached the hygroscopic coefficient, which was taken as the limit of availability. Water in some levels dropped to 2 per cent. below the hygroscopic coefficient.

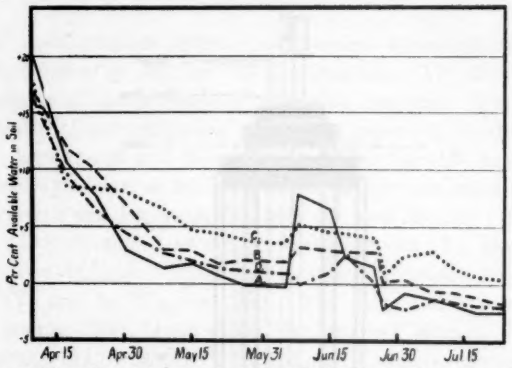

FIG. 2. Available soil moisture in the first 3 feet of upland prairie soil: *A*, at 0 to 6 inches depth; *B*, 6 to 12 inches; *C*, 12 to 24 inches; and *D*, 24 to 36 inches.

Relative humidity was always below 30 per cent. when samples were taken, and readings as low as 12–15 per cent. were encountered in July.

Table I includes a group of plants which succumbed in early summer to the severe drought. All the species blossom and fruit early in the season, and then most of them either dry or remain almost dormant in the shade of the tall grasses. Their osmotic pressures increased as the season progressed and the soil dried. While some of these grasses had ranges of 20 to 38 atmospheres, the forbs had usually a rather low range. Most of the early readings in this group were higher than average. In all cases the water contents varied inversely as the osmotic pressures.

Perhaps the most outstanding feature of the season's data is the fact that *Rosa arkansana, Liatris punctata, Kuhnia glutinosa, Psoralea floribunda,* and others, having roots 10 to 20 feet in depth (**21, 22**), did not show a great increase in osmotic pressure even when drought was most severe (table II). The maximum increase in this group was about 12 atmospheres, although some individuals showed almost no definitely increasing trend. The water content of the tissues decreased regularly although the decrease was less than that shown in more shallowly rooted species. Experiments have shown that prairie soils, although they may dry below the point of available water in the surface layers, are always moist at deeper layers (**23**).

Moderately deeply rooted upland plants, however, such as *Helianthus rigidus, Andropogon scoparius, Bouteloua gracilis, Solidago glaberrima,* and others, which extend from 3 to 9 feet deep, showed great increases in osmotic pressures as the soil dried (tables III and I). Most of the moderately deeply rooted grasses reached osmotic pressures of 35 or more atmospheres, and the xeric *Bouteloua gracilis* reached 60 atmospheres. Many forbs also showed

TABLE I

OSMOTIC PRESSURES AND WATER CONTENTS OF PLANTS THAT SUCCUMBED IN EARLY SUMMER EACH DRIED AT THE TIME OF LAST DETERMINATION OF ITS OSMOTIC PRESSURE

SPECIES	CRITERION	APR. 10	APR. 17	APR. 24	MAY 1	MAY 8	MAY 15	MAY 21	MAY 28
Allium mutabile	Per cent. water								
	Pressure		10.8	10.9			9.1		
Anemone caroliniana	Per cent. water								
	Pressure	17.3		18.5					
Antennaria campestris	Per cent. water								
	Pressure		9.9	12.4		18.2			
Astragalus crassicarpus	Per cent. water	84.0		82.3	78.5	74.0	72.9	71.0	69.2
	Pressure	12.4	10.9	13.2	13.3	15.4	15.6	17.9	18.7
Bouteloua gracilis	Per cent. water								37.7
	Pressure				21.6	25.9		43.5	59.5
Carex pennsylvanica	Per cent. water			64.6					
	Pressure	12.8	12.8	19.3			17.3		30.2
Cogswellia daucifolia	Per cent. water	79.7		79.5	77.1	77.3		70.1	63.5
	Pressure	14.4	11.5	14.6	16.1	16.1	16.6	17.0	20.2
Fragaria virginiana	Per cent. water								
	Pressure		12.0					14.9	
Koeleria cristata	Per cent. water								
	Pressure	15.8	16.4	17.4		24.1			35.0
Nothocalais cuspidata	Per cent. water								
	Pressure	12.8	13.2				15.2		
Poa pratensis	Per cent. water		72.5			57.4			
	Pressure	14.5				28.9	25.5		28.5
Sisyrinchium campestre	Per cent. water								
	Pressure				14.4				

increases of as much as 25 atmospheres. The range of osmotic pressures from early spring to midsummer was much greater in every species than that recorded by WALTER (**20**).

In this group also the water content of tissues varied inversely with the osmotic pressure and very great decreases were common. Many plants when growth ceased had only half as high a water content as had been recorded earlier.

It is evident from tables I–IV that the osmotic pressure and water content may be more or less characteristic of a species. Thus the values for an individual species may vary definitely from those of another in the same habitat. If one species has a higher osmotic pressure than another, in gen-

TABLE II

OSMOTIC PRESSURES AND WATER CONTENTS OF DEEPLY ROOTED UPLAND PLANTS. NONE SHOWED ABNORMAL DRYING

SPECIES	CRITERION	APR. 19	APR. 24	MAY 8	MAY 21	MAY 28	JUNE 8	JUNE 20	JULY 2	JULY 9	JULY 18	JULY 25	JULY 31
Amorpha canescens	Per cent. water			69.6	62.5	59.3	54.2	53.2	50.1	45.8	43.6	42.0	44.8
	Pressure		11.6	13.1	15.8	20.8	16.2	15.1	17.2	20.0	24.4	27.7	22.1
Baptisia leucophaea	Per cent. water				70.6	64.0	64.5	62.3	52.6				
	Pressure	8.6	10.8	12.0	11.4	14.8	16.0	16.7	17.3		24.0	20.6	
Kuhnia glutinosa	Per cent. water								71.2		69.0	63.5	68.3
	Pressure							11.5	12.1		12.4	15.0	11.5
Liatris punctata	Per cent. water						68.5	65.5	64.4	61.2	59.2	57.8	55.8
	Pressure					12.8	13.6	11.4	12.8	12.6	14.0	12.7	13.2
Psoralea floribunda	Per cent. water				71.5	71.1	67.2	63.5	61.7	59.2	55.1	56.2	
	Pressure		12.2	14.2	16.9	17.8	16.0	16.8	15.2	17.2	17.3	17.5	15.8
Rosa arkansana	Per cent. water						54.8	54.5		53.5	51.4		50.3
	Pressure		16.9		19.2		15.5	13.3		20.4	20.4		14.8

TABLE III

OSMOTIC PRESSURES AND WATER CONTENTS OF MODERATELY DEEPLY ROOTED UPLAND PLANTS, EACH OF WHICH DRIED AT TIME OF LAST DETERMINATION SHOWN

SPECIES	CRITERION	APR. 10	APR. 19	APR. 24	MAY 1	MAY 8	MAY 15	MAY 21	MAY 28	JUNE 1	JUNE 8	JUNE 15	JUNE 20	JUNE 25	JULY 2	JULY 9	JULY 18
Agropyron smithii	Per cent. water							51.5	49.2								
	Pressure	20.0			23.0		30.2	30.5	33.8		34.3		21.4			40.5	
Andropogon furcatus	Per cent. water							71.4			61.0		60.3			42.8	37.3
	Pressure			9.5		11.5	12.2	11.0	13.9		14.2	12.2	16.0			37.7	36.2
Andropogon nutans	Per cent. water					73.2		66.0		54.5	58.3	59.9	55.3	48.2		38.2	35.8
	Pressure			10.9		10.9	11.4	13.6	18.5	19.7	16.4	10.6	16.9	27.7		35.0	
Andropogon scoparius	Per cent. water			77.0	71.2	66.8		62.5	52.9	48.3	57.0	59.3	52.9	48.2	41.5	36.8	36.7
	Pressure		8.1	9.6	9.8	9.9	12.4	13.6	25.3	37.4	18.1	11.5	17.4	23.5	31.3	55.1	
Bouteloua curtipendula	Per cent. water																
	Pressure													26.0		40.4	
Helianthus rigidus	Per cent. water		82.0			78.7				66.3	71.4		64.5	53.4	48.0		
	Pressure	13.2				12.6		13.3	19.9	32.8	15.2		22.9	29.4	36.9		
Liatris scariosa	Per cent. water												60.9		42.2		
	Pressure										20.4		19.8		36.7		
Panicum scribnerianum	Per cent. water																
	Pressure			12.6							16.7		16.8				
Solidago glaberrima	Per cent. water							69.4		64.8	65.3			59.0		52.8	44.2
	Pressure	16.3		12.0			15.2	13.7	12.8	17.8	14.3	12.4	16.8	22.0		28.1	
Sporobolus heterolepis	Per cent. water			66.7				55.8	47.6		51.7	51.8	48.6	46.8	41.8	39.6	37.3
	Pressure		7.4	12.4	13.7		16.3	18.7	23.4		22.0	15.2	21.5	30.6	37.5		
Stipa spartea	Per cent. water				63.0		62.1	55.7	53.6		47.2					38.8	
	Pressure	15.6	18.2		16.9		16.4	17.3	18.4	24.9	21.8	20.0		25.5		32.7	

eral it can also be expected to have a lower water content. A high or low osmotic pressure may be characteristic of a species, but by extreme variation of available soil moisture the ranges of any two plants may be seen to overlap widely.

It is interesting to note that the prevernal and typically first layer plants, such as *Anemone caroliniana, Carex pennsylvanica,* and *Antennaria campestris* (table I), did not have the markedly lower osmotic pressures that were expected from their characteristically mesic habitat. This, however, may have been due in part to the unusually dry year.

The relationship between water content of the tissue and osmotic pressure should also be noted. In most cases shown in tables I-IV the water content decreased regularly as the season progressed, and, while the water content may have increased during rainy periods, it never responded as much proportionately as did the osmotic pressure. As the plants matured some cells may have dried completely, and having no sap, they would not influence the osmotic pressure, but having dry matter they would tend to decrease the percentage of water in the tissue. There are probably some physiological changes also, such as thickening of the cell walls, which are characteristic of older plants, that may in part account for this fact. As the season

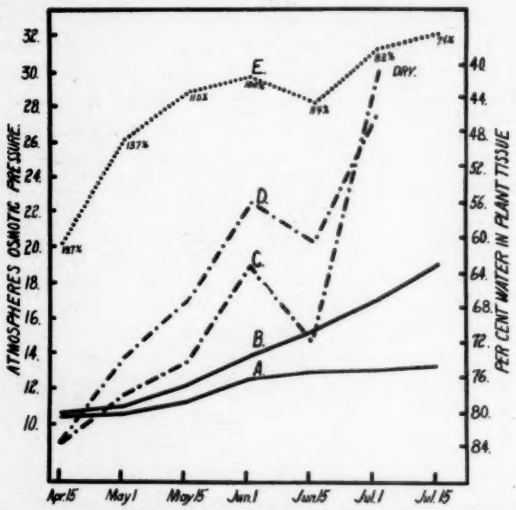

FIG. 3. Graphs of average osmotic pressure (*A*) of *Aster salicifolius, Helianthus grosseserratus, Panicum virgatum, Solidago altissima,* and *Spartina michauxiana,* all of lowland, and water content of their tissues (*B*). Water content (*C*) of *Andropogon scoparius, Baptisia leucophaea, Helianthus rigidus, Solidago glaberrima,* and *Sporobolus heterolepis,* all of upland, and their osmotic pressure (*D*). Average total water content (*E*) of first 3 feet of upland soil.

progressed, therefore, the curve of dry matter tended to deviate steadily from the curve of osmotic pressure. This fact is well shown in lowland plants where little variation in osmotic pressure occurred (graphs A and B, fig. 3).

The small seasonal change in osmotic pressure and water contents of plants listed in table II indicates that their long roots enable them to obtain a sufficient water supply at all times, and that they are the truly drought resistant plants of the prairie. The same tendency is shown in typical lowland plants such as *Helianthus grosseserratus, Aster salicifolius, Solidago altissima, Panicum virgatum,* and others (table IV), for these plants are able to obtain sufficient water because of its greater abundance in the lowland soil. The water table in the lowland seldom falls below 4 feet and water is probably always available below the first 6 inches. Many of these lowland plants show no tendency to increase their osmotic pressure more than one or two atmospheres.

This lack of any very great increase of osmotic pressure in plants of the lowland is further illustrated in figure 3, which shows comparative seasonal curves of five typical lowland and five typical upland plants. The upland plants showed a great increase in pressure as the season progressed while the lowland plants showed a comparatively small increase. The two grasses in each group showed greater increases than did the forbs. That lack of water caused the increase in the upland plants is further borne out in this graph by the fact that their pressures decreased abruptly after the rains of early June. That lowland plants do not have an osmotic pressure typically lower than upland plants, except during times of stress on the upland, is shown by the readings on April 15 at which time the upland plants had the lower average pressure owing to the plentiful soil moisture. Osmotic pressures as an index to site or habitat should therefore be used only during drier seasons.

Responses of deeply and shallowly rooted plants

The same lack of response of plants having access to water is shown by samples of the same species on the same day from both upland and lowland habitats. Figure 4 shows that, in both lowland and upland habitats, the deepest rooted species such as *Rosa arkansana, Liatris punctata,* and *Kuhnia glutinosa,* which penetrate to a depth of 16 to 21 feet, vary but little and sometimes insignificantly both in osmotic value and in water content. In the same two habitats, however, plants such as *Andropogon furcatus, Helianthus rigidus,* and *Solidago glaberrima,* whose maximum root penetration varies only from 7 to 9 feet, show a great difference in both water content of tissues and osmotic pressure. Experiments have shown intermediate values for these plants on midslopes. This shows that with shallower rooted species the dryness of the habitat is indicated by the osmotic pressure and the water

TABLE IV

OSMOTIC PRESSURES AND WATER CONTENTS OF IMPORTANT LOWLAND SPECIES WHICH NORMALLY HAVE AVAILABLE WATER AT ALL TIMES. MOST SPECIES SHOWED NO EFFECTS OF THE DROUGHT

SPECIES	CRITERION	APR. 10	APR. 17	MAY 1	MAY 15	MAY 24	JUNE 1	JUNE 13	JUNE 25	JUL. 2	JUL. 9	JUL. 25	JUL. 31
Aster salicifolius	Per cent. water	83.5	84.2	82.4	80.3	77.6				69.9			65.2
	Pressure	13.0	11.3	9.1	11.4	12.5		12.0	12.7	11.8	11.8	11.4	12.8
Carex vulpinoidea	Per cent. water		69.0	69.0		60.9						33.2	
	Pressure	12.7	13.9	14.5		18.8			19.9			41.1	Dry
Glycyrrhiza lepidota	Per cent. water								65.8			60.0	60.0
	Pressure				13.8		14.5	16.2	16.0			18.8	16.1
Helianthus grosseserratus	Per cent. water			83.6	81.5	80.6		77.0	74.8	76.0	72.0	70.7	71.0
	Pressure		9.2	10.8	11.8	13.2		12.8	12.2	12.6	12.5	11.6	11.5
Panicum virgatum	Per cent. water								65.0		57.5	57.4	56.2
	Pressure			10.1	12.8	10.6	10.6	11.3	10.7		12.0	13.7	12.8
Phalaris arundinacea	Per cent. water				74.4	74.2	72.1	69.9	64.4			59.9	
	Pressure	13.8	14.4	19.2	16.3	16.3	17.4	18.2	17.3			21.5	
Rhus glabra	Per cent. water									78.0			
	Pressure		11.2							10.9			
Solidago altissima	Per cent. water	84.8	82.3	81.1	81.3	74.1		70.6	71.8	67.4	66.8	64.2	64.4
	Pressure	10.3	9.0	10.7	10.2	11.4		12.4	11.3	10.9	10.8	13.2	12.1
Spartina michauxiana	Per cent. water		76.3	72.5	66.3	63.4	58.7	61.2	60.0	55.4	54.7	53.2	
	Pressure	15.7	12.6	12.7	13.2	11.3	16.8	17.9	15.0	19.8	19.7	22.1	
Typha latifolia	Per cent. water		87.0		87.8			79.7	76.7			71.8	
	Pressure		6.2		8.3			10.8	11.8			18.7	

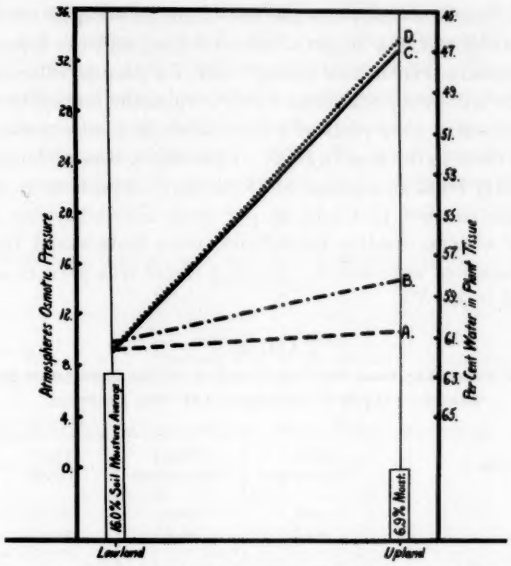

FIG. 4. Average osmotic values of the same species growing on both upland and lowland, from samples collected at the same time. Average osmotic pressure (*A*) of the deeply rooted *Liatris punctata, Kuhnia glutinosa,* and *Rosa arkansana,* and their average water content (*B*). Average osmotic pressure (*C*) of the moderately rooted *Andropogon furcatus, Helianthus rigidus,* and *Solidago glaberrima,* and their average water content (*D*).

content of the tissue. It further shows that in all cases the water available to the plant is indicated both by the osmotic pressure and the water content of the tissue, whether this availability be due to depth and efficiency of the root system or to an abundance of water.

These results may serve to explain the many conflicting reports as to the value of osmotic pressure as a criterion of site. They show that deeply rooted plants should not be used as indicators and that only during times of stress are differences in site expressed. When judiciously employed, however, osmotic pressure is an excellent index.

HUMIDITY AND SOIL MOISTURE AS FACTORS INFLUENCING OSMOTIC PRESSURE

An experiment was conducted to determine the relative influence of humidity and soil moisture. Four closely adjacent plots with similar vegetation, each about 2 meters square, were selected. Two were watered by means of sprinkling during a period of three days. This wet the soil to a depth of 3 feet and increased the average soil moisture from 11.9 to 19.1 per

cent. One of these watered plots and one of the unwatered ones were walled in with stiff cardboard to a height of about 3 feet, and the tops covered with muslin. The muslin transmitted enough light for photosynthesis but afforded sufficient shade and protection from wind to raise the humidity considerably. Inside the enclosures were pans of water which had saturated muslin strips running from them to the muslin roof. This device was sufficient to raise the relative humidity from an average of 33 per cent. outside to an average of 51 per cent. inside the wet plot and 46 per cent. inside the dry plot. These humidities, or at least similar humidities, were maintained for at least 48 hours before samples were taken. No free water was present on the plants when collected (table V).

TABLE V

AVERAGE OSMOTIC PRESSURES FROM TWO EXPERIMENTS DESIGNED TO SHOW RELATIONSHIP OF OSMOTIC VALUES TO HUMIDITY AND SOIL MOISTURE

SPECIES	DRY, NON-HUMID	WET, NON-HUMID	DRY, HUMID	WET, HUMID
	atm.	*atm.*	*atm.*	*atm.*
Stipa spartea	24.9	20.6	18.9	19.2
Sporobolus heterolepis	15.3	14.8	12.0	10.7
Amorpha canescens	16.5	16.3	13.5	13.2
Solidago glaberrima	15.1	14.6	13.7	12.8
Andropogon nutans	13.7	13.6	12.6	12.1
Andropogon scoparius	15.7	11.5	12.6	9.8
Average	16.21	14.75	13.57	12.57

It may be seen from table V that both increased humidity and increased soil moisture decreased the osmotic values. When both were increased the effect was considerably greater than when only one was augmented.

It is of interest that the osmotic pressures of the deeply rooted *Amorpha canescens*, which absorbs very little in the surface 2 feet, were scarcely affected by watering the soil. As would be expected, however, they were decreased by increasing the humidity.

In all but one species, *Andropogon scoparius*, the increase of humidity alone gave a greater change than did increase of water content of the soil alone. This fact does not necessarily indicate, however, that soil moisture is a less important factor in influencing osmotic pressure than is humidity. This soil moisture was necessarily mostly near the surface, and at best the indi-

cation would be only that this particular humidity change was more important than the soil moisture change. It does, nevertheless, show the importance of humidity as a factor affecting osmotic value. The osmotic value decreases as the plant tissue increases its water, whether this increase is due to greater absorption or to decreased transpiration. This relationship to water content of tissues is shown in table VI, which gives data for those plants which were sufficiently abundant to yield a water content sample.

TABLE VI

WATER CONTENT OF PLANTS FROM AREAS ON WHICH HUMIDITY AND WATER CONTENT OF SOIL WERE VARIED

SPECIES	DRY, NON-HUMID	WET, NON-HUMID	DRY, HUMID	WET, HUMID
	%	%	%	%
Sporobolus heterolepis	51.8	53.5	.....	56.0
Amorpha canescens	52.3	53.2	54.8	54.3
Solidago glaberrima	64.8	.....	65.6	70.7
Andropogon nutans	55.2	55.2	.....	60.5
Andropogon scoparius	54.9	62.1	61.0	63.6

These water content values show the same sequence as do the osmotic pressures, and strongly indicate that the osmotic change was due solely to change in water content and not to any change in photosynthetic or other physiological processes. This probably was due to the extremely hot dry weather, which prohibited stomatal opening. It is concluded, therefore, that both humidity and soil moisture are important factors, and that both act through the medium of the water content of the tissues.

DAILY CYCLE OF OSMOTIC PRESSURE AND WATER CONTENT

The daily trend of osmotic pressure in *Andropogon scoparius,* the dominant upland prairie grass, was determined. At the time of the investigation the soil was very dry and the humidity correspondingly low. All grass leaves were folded and did not open even during the night. It seems probable that the stomata did not open during the experiment and that no carbon dioxide was absorbed for photosynthesis. We may conclude, therefore, that the cyclic change was due largely to increase and decrease of the water stress. In order to eliminate the factor of soil heterogeneity, twelve plots were staked off and each of the twelve samples was composed of equal parts from each of the plots. Samples for both water content and osmotic

pressure were taken in duplicate every 2 hours for a period of 24 hours. Figure 5 shows the graphs for these two values together with the temperature and humidity. During this period the osmotic pressures were rising rapidly,

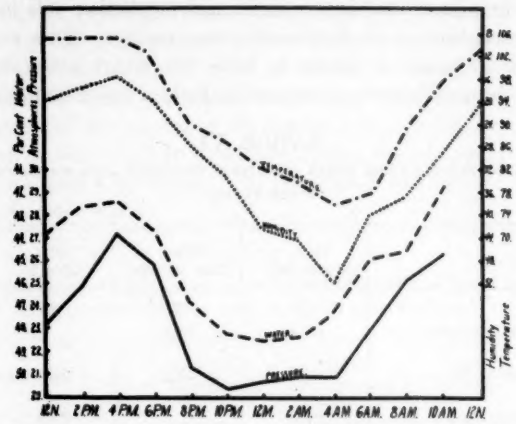

FIG. 5. Daily cycle of water content and osmotic pressure of *Andropogon scoparius* during extreme drought. Relative humidity and air temperature are also given.

and it is probable that on the second day they rose considerably above those on the first day. The graph is much as would be expected, except for the tendency of the osmotic pressure to reach a minimum early in the night and to maintain that minimum until early morning. This may indicate that the osmotic pressure of the plant had decreased to the same value as that of the soil water, and that, in such a case, the value for the plant sap could decrease no further despite the fact that the humidity had ceased to make so great a demand upon the water of the plant. The decrease in the water content of the plant tissues from 2 to 4 A.M. is not clear.

These graphs present further evidence of the importance of humidity as a factor affecting osmotic pressure. They show definitely that a daily curve may be effected by moisture relations as well as by photosynthesis. Since soil moisture varied but little during the experiment and there was probably no photosynthesis, the changes may be attributed to variations in humidity.

A similar daily cycle test was made with the lowland sunflower, *Helianthus grosseserratus,* but in this case, where soil moisture was plentiful, the daily change did not exceed two atmospheres' pressure. Here also the maximum pressure was obtained during the hot afternoon and the minimum during the more humid night. The data show that where soil moisture was sufficient to supply the demands of the atmosphere the daily range was much less than that found in the drier uplands.

Comparative values of roots and tops

Experiments were conducted with *Panicum virgatum* in order to ascertain the gradients of osmotic pressure and water content between the tops and roots. Rhizomes were planted in soil consisting of nine parts of sand and one part of loam. They were allowed to grow until the time of flowering, when the large wooden containers were opened and the roots of one plant were quickly shaken free of adhering sand and frozen for osmotic pressure determination. The osmotic pressure of the top of the plant was also determined. A second plant was used for ascertaining the water content of both roots and top. So far as possible the roots were shaken free from sand before drying. Water content of the sand-soil mixture was also determined so that calculations of the water held by the sand adhering to the roots might be made. When the sample of roots was dry, it was reweighed to get total water loss. It was then washed free from all sand and soil. The weight of the roots and the weight of this adhering sand and soil were next determined. The part of water that had been lost by the adhering soil and the part lost by the roots were then obtained. Finally the water content of the roots was calculated. The water contents of the roots and top were 74.5 and 59.9 per cent. respectively, and the osmotic pressures 8.43 and 13.84 A. Thus the osmotic pressure was greater in the top of the plant, and the water content varied inversely as the pressure.

Effect of age of tissue on osmotic pressure and water content

Late in the season, when most plants had reached a very high osmotic pressure, a plot was watered artificially for a period of three weeks. Some of the area was clipped prior to watering to allow new vegetation to be produced while other parts were left undisturbed in order to preserve the old vegetation. Four species gave enough new growth to permit a test in which the tissue was two weeks or less of age. A determination was also made on old growth in which only mature tissues were used. The results are shown in table VII. It may be seen that in all cases the older tissue had a higher osmotic pressure, despite the fact that the soil moisture was identical in each case. In the two plants tested the water contents varied inversely as the osmotic pressures, as would be expected. This experiment indicates that osmotic pressure increased as the tissue aged regardless of water content of the soil, although in some species, such as the grasses, the increase was very small.

It is of interest to note that on the watered plot the old tissues in many plants decreased their osmotic pressures to values such as were recorded in early spring, and the water content in the plant tissue rose to almost as high a figure as had been recorded early in the season. The average soil moisture

TABLE VII

OSMOTIC PRESSURE OF NEW AND OLD TISSUES UNDER SAME SOIL MOISTURE CONDITIONS

Species	New tissue		Old tissue	
	Osmotic pressure	Water content	Osmotic pressure	Water content
	atm.	%	*atm.*	%
Amorpha canescens	12.88	.....	19.60	43.5
Andropogon scoparius	9.39	71.3	9.75	68.5
Solidago glaberrima	10.48	75.7	16.72	58.2
Sporobolus heterolepis	12.76	.....	13.60	62.3

in the first 5 feet was only 10 per cent., about 3 per cent. above the hygroscopic coefficient, although the surface soil contained 19 per cent. The generally high humidity at this time likewise was conducive to low osmotic pressures. The deeper rooted species showed much less response than did the shallower rooted ones. Table VIII shows a few of these values with previous high and low readings for comparison.

It is evident from table VIII that the osmotic pressure can be reduced to the spring value, or very nearly so, with an increase in soil moisture. Old tissue tends to be slightly higher in osmotic value than new tissue. As has already been pointed out, however, the water content of the tissue can never be brought back to the high value of spring because of permanent physiological changes and possibly death of some cells.

TABLE VIII

OSMOTIC PRESSURES AND WATER CONTENT VALUES ATTAINED ON A WATERED PLOT, WITH PREVIOUS HIGH AND LOW READINGS

Species	Watered plot		Late summer		Early spring	
	Osmotic pressure	Water content	Osmotic pressure	Water content	Osmotic pressure	Water content
	atm.	%	*atm.*	%	*atm.*	%
Amorpha canescens	19.60	43.5	27.75	42.0	11.68	69.6
Andropogon furcatus	10.36	.....	35.04	37.6	10.96	77.0
Andropogon nutans	9.99	71.4	37.76	35.8	9.51	73.2
Andropogon scoparius	9.75	68.5	37.47	36.7	8.19	77.0
Bouteloua gracilis	12.88	.....	59.50	37.7	.....	.....
Helianthus rigidus	11.28	.....	36.95	48.0	9.51	82.0
Liatris punctata	11.80	59.8	14.08	57.8	11.44	68.5
Solidago glaberrima	16.72	58.2	28.46	44.2	11.04	75.2
Sporobolus heterolepis	13.60	62.3	37.54	37.3	7.47	66.7
Stipa spartea	16.72	.....	32.77	38.8	15.64	63.0

Discussion

Osmotic pressures and water content of tissues closely parallel each other, and both are highly indicative of the availability of water. The fact that one species may develop a high osmotic pressure while that of another remains low implies that the first is suffering from water shortage while the second is not. Thus the high pressure is deemed to be a result of drought and not an adaptation to it. The plant's ability to increase its osmotic pressure and thus keep the osmotic gradient in its favor is doubtless a valuable feature during periods of stress. It is rendered less valuable, however, by the fact that any possible increase will enable the plant to absorb water from a soil but little drier than that from which it could extract water without increase in pressure. This is due to the fact that the osmotic pressure of the soil solution, which is normally below that of even the most mesic plants, as it approaches the point of non-availability rises very rapidly to extremely high values not attainable by plants (**19**, p. 25). It is also possible that slowness of water movement may cause wilting even while the osmotic pressure of the plant is still greater than that of the soil solution (**19**). In this case osmotic pressure of the sap would never be a factor involved in drought resistance.

Colloidal imbibition is capable of exerting much greater force than is osmotic pressure and is probably the more important factor acting to secure water from a dry soil (**19**).

These studies emphasize the fact that the drought resistance of prairie plants is due largely to their extremely deep and efficient root systems.

Summary

1. Osmotic pressure is regarded as an expression of the environment, indicating the relative forces with which the soil supplies water to the plant and the air removes it through transpiration.

2. As the soil dried with the progress of the season the osmotic pressure of all prairie plants studied increased. This increase was frequently 30 or more atmospheres among moderately deeply rooted upland plants. Among deeply rooted upland plants and plants growing in low moist habitats the increase was usually only 2 to 10 atmospheres.

3. As the soil dried, the water content of the tissue very regularly decreased, although the change was much less marked in lowland plants and in those having very deep roots.

4. Water content of plant tissue is a rather exact indicator of osmotic pressure, plants with high pressures usually having low water content and plants with low pressures usually having high water content.

5. Osmotic pressure of the sap and water content of the tissue responded

readily to increases in soil moisture, but the response was much less in the case of water content of tissue.

6. Osmotic pressure and water content of the tissue, except in very deeply rooted plants, are both excellent criteria of the water content of the habitat.

7. Variation of both humidity and soil moisture indicated that these factors had a great influence on both osmotic pressure and water content of tissues. Minimum osmotic pressure and maximum water content of tissues were attained with a high humidity and a high soil moisture.

8. Determinations of both osmotic pressure and water content of *Andropogon scoparius* every two hours during a day and a night showed a reverse correlation between osmotic pressure and humidity, and a direct correlation between humidity and water content of the tissues.

9. Similar determinations on a lowland species, *Helianthus grosseserratus*, rooted in moist soil, showed much less daily variation.

10. Roots of *Panicum virgatum* had a lower osmotic pressure and a higher water content than the tops.

11. New growth of plant tissue had a lower osmotic pressure and a higher water content than old growth.

12. Artificial watering in late summer lowered osmotic pressure and increased water content of tissues. The values approached those of spring.

13. Soil moisture and humidity are considered the major factors affecting osmotic pressure.

The writer wishes to express sincere appreciation to Dr. J. E. WEAVER, under whose direction this work was done.

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KINETICS OF AN INTRACELLULAR SYSTEM FOR RESPIRATION AND BIOELECTRIC POTENTIAL AT FLUX EQUILIBRIUM

GORDON MARSH

(WITH FOUR FIGURES)

Introduction

The oxidation-reduction theory of bioelectric currents formulated by LUND (**14**) has received much experimental confirmation (**16, 17**). The theory assumes the normal aerobic respiratory process in the living cell to give rise to the electromotively active materials, as follows:

$$\text{(a)}\ X \rightarrow AH_2$$
$$\text{(b)}\ AH_2 + O \rightarrow A + H_2O$$
$$\text{(c)}\ A \rightarrow Y \rightarrow CO_2$$

X is a precursor substance; AH_2 and A are the reductant and oxidant, respectively, of the electromotive material.

If we assume the dissociation $AH_2 \rightarrow A + 2H^+ + 2e$ we may write

$$[e] = \sqrt{\frac{K\,[AH_2]}{[H^+]^2\,[A]}}$$

where [e] is the electron concentration of the solution. The equation for the electromotive force developed at a locus (phase boundary) within the living cell may then be written:[1]

$$E_h = E_o - \frac{RT}{2F} \ln \frac{[A]}{[AH_2]}$$

where E_h is the potential referred to the hydrogen electrode, E_o a constant (containing $\frac{RT}{2F} \ln \frac{[H^+]^2}{K}$). The difference of potential across the living cell is assumed to consist of the difference between the oppositely oriented potentials of at least two loci, or

$$E_p = E_{h_1} - E_{h_2}$$

The E.M.F. measured across a tissue will be the algebraic sum of the polarity potentials of all the cells included in the measuring circuit, or

[1] The convention of sign is that of LEWIS and RANDALL (**12**, pp. 389, 402), which is the simple negative of the more commonly used European convention (**20**, p. 465). An increase in the ratio $\frac{[A]}{[AH_2]}$ increases the negative value of E_h. The European convention, however, has been followed in expressing the measured E.M.F. of cells and tissues. In consequence, the locus of high negative potential according to the equation is *measured* as the point of high positive potential. This has led to LUND'S formal error of statement that the value of the ratio is smaller at that locus which is positive in the measuring circuit.

$$E = \Sigma E_p$$

For a given rate of oxygen uptake the ratio $\frac{[A]}{[AH_2]}$ will possess a constant value, a condition termed by LUND a flux equilibrium. The E.M.F. will vary with oxygen pressure and other factors influencing the rate of metabolism, although the variation is not defined precisely in LUND'S formulation (in part due to the inclusion of an oxygen concentration term in the equation).

The present paper supplies a quantitative connection between the velocity of the oxidative reactions and the measured E.M.F. of a cell or tissue. Certain properties of the intracellular system thus emerge which are not otherwise obvious. The system also yields a satisfactory equation linking the rate of oxygen consumption to oxygen pressure.

At the risk of promoting a sterile controversy, it seems necessary to introduce the question of intracellular electrodes. The one objection urged against the oxidation-reduction theory of bioelectric phenomena is that metallic electrodes are absent from the cell (**2**). This argument is entirely *a priori*. It should be unnecessary to point out that the platinum (or other metal) electrode is *not* essential to the formulation of equations expressing the potential of an electrochemical system, nor to its manifestation (**6**, p. 378). "Metallic" conduction is not restricted to "metals," and a normal cell structure possessing the *essential properties* of an electrode is physically possible.

LUND'S theory assumes the existence of such structures (or interfaces). Aside from its implications there is no evidence upon this point.[2] In view of the unanimity of the experimental support which the theory has received and its power to unite an extensive body of data into a coherent whole, the writer prefers to accept the existence of intracellular electrodes rather than to dismiss as irrelevant the evidence which LUND and his coworkers have amassed.

Relation of E_h to respiratory velocity at flux equilibrium

The steps in the oxidative reaction may be amended[3] to read:

[2] An attempt to provide direct evidence (21) has proved abortive. The color change reported at the borders of *Tradescantia* cells proved to be due primarily to retraction of the protoplast from the cell wall, not to a reversible pH change. The conclusion that conductors of the first class exist within the cell is unsupported (see also BLINKS, 3).

[3] LUND'S equation (b) was not intended to specify the intervention of atomic oxygen. Consequently no great violence is done in altering the reaction to equation (2) above, while the mathematical treatment may be considerably simplified. The extra oxygen atom is here combined in A; for purposes of formulation the reaction might be written in any of a number of other ways. Until our knowledge of the specific chemical details of the respiratory process is greatly enhanced, the dynamics of the system cannot with profit be elaborated beyond some simple formulation like the present one.

$$X \rightleftarrows AH_2 \quad (1)$$
$$AH_2 + O_2 \rightarrow A + H_2O \quad (2)$$
$$A \rightarrow y \rightarrow CO_2 \quad (3)$$

Because of the existence of (a) "basal metabolism" and (b) an asymptote to the oxygen consumption:oxygen pressure curves (equation (15) below), we may write $[X] = C$. (C varies with the nutritional state, but appears constant for a given state.) The number of equivalents (per liter) of X transformed irreversibly in time t will then be $\frac{-dx}{dt} = k_1C = K_1$. At flux equilibrium the velocities of production and removal of AH_2 and A and the velocity of oxygen consumption will all equal K_1, unless respiration be primarily determined by rate of diffusion of oxygen, a condition which theoretical and experimental investigations indicate to be non-existent, or extremely rare (**11, 9**). Under these conditions the E.M.F. at a locus will vary with oxygen pressure but the rate of oxygen consumption will be constant. Since, experimentally, consumption does vary with pressure, $X \rightleftarrows AH_2$ must be reversible.

Let $[X] = C$. Then k_1C = the tendency of X to form AH_2, and $k_2[AH_2]$ = the tendency of AH_2 to form X. The number of equivalents (per liter) of X changing to AH_2 in time t will be:

$$\frac{-dx}{dt} = k_1C - k_2[AH_2]$$

This is also the rate of production of AH_2 due to reaction (1), or

$$\frac{d[AH_2]}{dt} = k_1C - k_2[AH_2] \quad (4)$$

The rate of disappearance of AH_2 according to reaction (2) may be written

$$\frac{-d[AH_2]}{dt} = k_3P_c[AH_2] \quad (5)$$

where k_3 is the velocity constant of reaction (2) and P_c is the flux equilibrium oxygen tension at a locus within the cell.

At flux equilibrium (4) = (5), whence

$$[AH_2] = \frac{k_1C}{k_2 + k_3P_c} \quad (6)$$

The rate of disappearance of AH_2 equals the rate of production of A. Combining (6) and (5)

$$\frac{d[A]}{dt} = \frac{k_3k_1CP_c}{k_2 + k_3P_c} \quad (7)$$

The rate of disappearance of A due to reaction (3) is then

$$\frac{-d[A]}{dt} = k_4[A] \quad (8)$$

where k_4 is the velocity constant of reaction (3). As before, at flux equilibrium (7) = (8), and

$$[A] = \frac{k_3 k_1 C P_c}{k_4 (k_2 + k_3 P_c)} \tag{9}$$

The ratio of concentrations of oxidant and reductant at a locus (at flux equilibrium) will then be

$$\frac{[A]}{[AH_2]} = \frac{k_3 P_c}{k_4} \tag{10}$$

and the electromotive force developed at a locus becomes

$$E_h = E'_o - \frac{RT}{2F} \ln P_c \tag{11}$$

$\frac{-RT}{2F} \ln \frac{k_3}{k_4}$ is included in the constant E'_o.

P_c may be expressed as a function of the velocity constants of the reactions and the oxygen pressure of the medium surrounding the living cell. We may write the rate of diffusion of oxygen into a locus, in accordance with Fick's law, as

$$\frac{do}{dt} = k_o (P - P_c) \tag{12}$$

where P is the oxygen tension of the medium in contact with the cell and k_o is a diffusion constant for oxygen. The rate of oxygen consumption at a locus $\left(\frac{-do}{dt}\right)$ may be expressed by (7). At flux equilibrium the rate of diffusion equals the rate of consumption; equating (7) and (12) we obtain the expression

$$k_o k_3 P_c^2 + (k_3 k_1 C + k_o k_2 - k_o k_3 P) P_c - k_o k_2 P = 0$$

whose roots are

$$P_c = \frac{k_o k_3 P - k_3 k_1 C - k_o k_2 \pm \sqrt{(k_1 k_3 C + k_o k_2)^2 + P^2 k_o^2 k_3^2 - 2P(k_o k_1 k_3^2 C - k_o^2 k_2 k_3)}}{2 k_o k_3}$$

This may be simplified by writing

$$\frac{k_1 k_3 C + k_o k_2}{k_o k_3} = a, \text{ and } \frac{k_1 k_3 C - k_o k_2}{k_o k_3} = b$$

The constants are all positive in sign as written, and not zero. When $P = 0$, $P_c = 0$, hence

$$\frac{-a \pm \sqrt{a^2}}{2} = 0$$

Only the positive root satisfies this condition. Therefore

$$P_c = \frac{P - a + \sqrt{a^2 + P^2 - 2Pb}}{2} \tag{13}$$

and

$$E_h = E'_o - \frac{RT}{2F} \ln \frac{P - a + \sqrt{a^2 + P^2 - 2Pb}}{2} \tag{14}$$

Figure 1 shows the variation of P_c with P for certain values of the con-

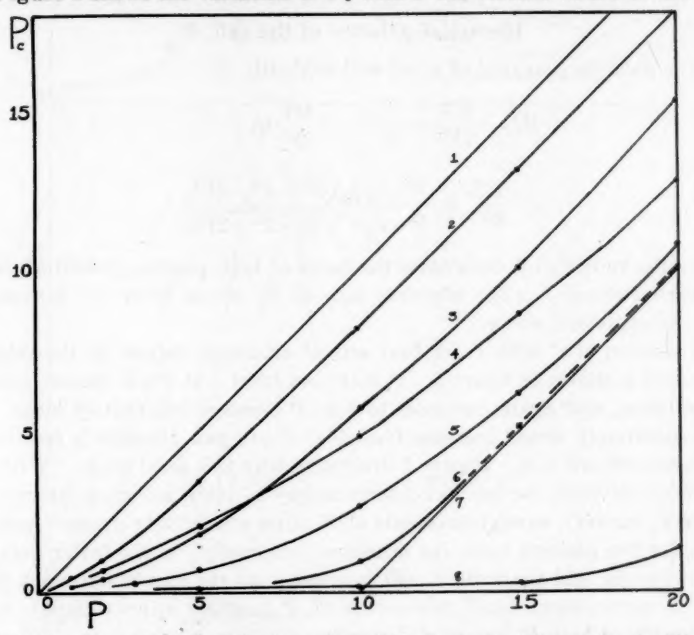

FIG. 1. P_c *vs.* P for the following values of the constants: (2) a = 3, b = 1; (3) a = 6, b = 4; (4) a = 15, b = 5; (5) a = 11, b = 9; (6) a = 10.1, b = 9.9; (8) a = 20.1, b = 19.9. (1) is the line $P_c = P$; (7) is the line $P_c = P - \frac{a+b}{2} = P - 10$, to which (4), (5), and (6) are asymptotic.

stants. $P_c = P$ at $P = 0$; from zero P_c increases with P to become asymptotic to $P_c = P - \frac{a+b}{2}$. The smaller the value of $a - b$ the more rapidly the curve approaches the asymptote, as may be seen in curves (4), (5), and (6). The reference line $P_c = P$, curve 1, is included in the figure to show how the flux equilibrium oxygen tension, P_c, differs from the external oxygen pressure, P, for different sets of values of the constants. The larger

the sum $a+b$ and the smaller the difference $a-b$ the more widely does P_c depart from P.

E_h, correspondingly, increases with increasing P (in harmony, it might be noted, with biological redox potential measurements). The locus in the living cell becomes, under this treatment, a modified oxygen electrode, and the ratio $\frac{[A]}{[AH_2]}$ cannot be combined with P (or [0]) in the equation for E_h, as done by LUND (**16**, p. 244).

Electrical polarity of the cell, E_p

The polarity potential of a cell will evidently be

$$E_p = \frac{-RT}{2F} \ln \frac{P_{c_1}}{P_{c_2}} = \frac{-RT}{2F} \ln r'$$

$$= \frac{-RT}{2F} \ln \frac{P - a_1 + \sqrt{a^2 + P^2 - 2Pb_1}}{P - a_2 + \sqrt{a_2^2 + P^2 - 2Pb_2}}$$

where the subscript 1 designates the locus of high positive potential in the measuring circuit. (The negative sign of E_p comes from the convention used, as explained above.)

Variation of r′ with P for four sets of arbitrary values[4] of the velocity constants is shown in figure 2. r′ increases from 1 at $P = 0$, passes through a maximum, and again decreases to 1 as P becomes indefinitely large. E_p correspondingly would increase from 0 at $P = 0$, pass through a maximum, and again return to 0. Figure 1 illustrates why this must be so. With the difference between the two loci determined by k_1 (thus avoiding intersection of the P_c curves), equal increments of P cause a relatively greater increase in P_c for the positive locus (as measured externally) in the lower portions of the curves, and the ratio r′ will increase. As the curves approach their parallel asymptotes, equal increments of P produce approximately equal increments in both P_c's, and r′ decreases.

The position of the maximum on the P axis and the absolute value of the maximum are functions of the values of the constants. No general solution for either position or value has been found. Differentiating r′ with respect to P yields an equation which is too involved to admit of solution by ordinary methods. It can only be demonstrated that substitution of appropriate values for the velocity constants furnishes numerical results which are in harmony with the experimental facts.

For the ascending limb of the curve the polarity potential increases with increasing P. The position of the maximum for systems whose variation

[4] For purposes of illustration it is assumed that the difference between two loci is solely determined by the velocity constant k_1, which is smaller at the locus of greater E_h. No significance is to be attached to such a choice of constants.

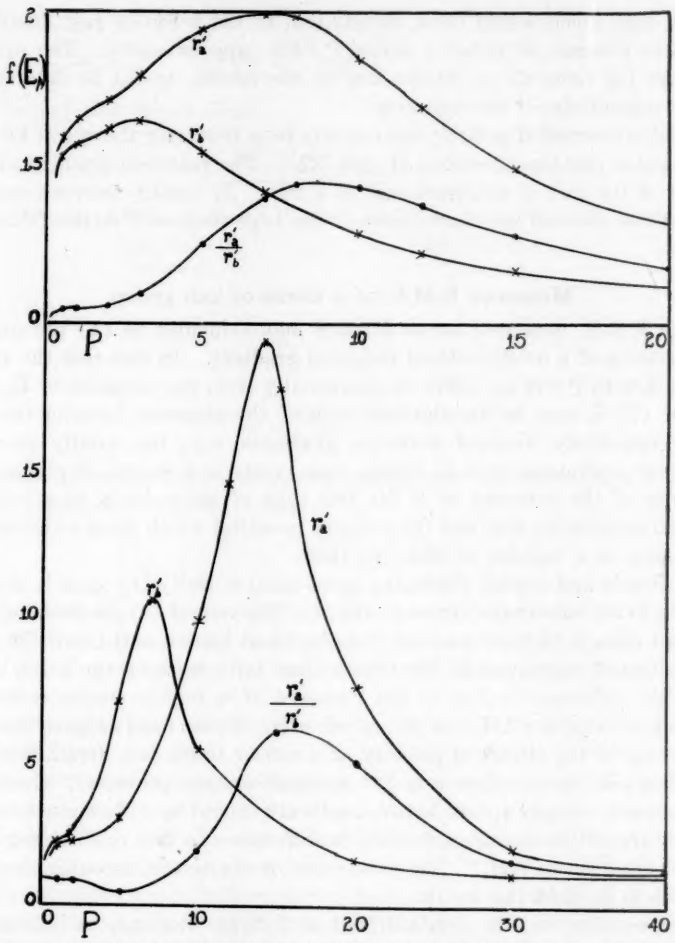

FIG. 2. Variables of the equations for E_p and E plotted against oxygen pressure. $r'_a = f(E_p)$ for apical cell; $r'_b = f(E_p)$ for basal cell. $r'_a/r'_b = f(E)$, the E.M.F. being oppositely oriented in the two cells. B shows reversal of apico-basal polarity at low values of P.

with P has thus far been investigated (frog skin, **15, 8**; onion root, **22**) evidently lies outside the range of oxygen pressures used.

If the difference between the two loci giving rise to E_p involves one or more constants in addition to k_1 and C, the P_c curves may intersect (curves (3) and (4), fig. 1). Let P_{c_1} follow curve (3) and P_{c_2} follow curve (4).

E_p for such a cell would show, in addition to the behavior just described, complete reversal of polarity below $P = 6.5$ (approximate). The critical pressure for reversal, or intersection of the curves, would be determined by the magnitudes of the constants.

Such a reversal of polarity has recently been found for the apical 1.5 mm. of the onion root tip exposed to H_2 gas (**22**). The potential gradient of this region of the root is unidirectional as a rule. If basally oriented regions existed the reversal may have been of the type discussed in the following section.

Measured E.M.F. of a tissue or cell group

The E.M.F. measured across a tissue may evidently be (1) the sum of the elements of a unidirectional potential gradient. In this case the variation of E with P will not differ fundamentally from the variation of E_p with P. Or (2) E may be the algebraic sum of the elements forming two (or more) oppositely directed potential gradients, *e.g.*, the axially growing organs or organisms such as *Obelia* stem, roots and shoots of plants, etc. Analysis of the behavior of E for this type of tissue leads to a relation between respiratory rate and the polarity potential which gives a theoretical foundation to a number of observed facts.

In tissues and organs possessing apico-basal polarity the apex is electropositive to the base in the external circuit. The rate of oxygen consumption per unit mass is higher for apical than for basal tissues, and LUND (**16**) has demonstrated unequivocally for *Obelia* (and later work on the onion root) that this difference is due to the presence of a higher concentration of oxidizable material (AH_2) in the apical cells. These facts suggest that the magnitude of the electrical polarity of a cell or tissue is a direct function of its oxygen consumption rate (at constant oxygen pressure), where the differences in oxygen uptake between cells are caused by differences in value of their respective constants, leading to differences in flux equilibrium level of concentration of AH_2.[5] The properties of the system as outlined above are such as to yield this result.

The relation may be demonstrated as follows: Assume, as before, the difference between two loci in the cell to be determined by k_1. Let the differences in level of $[AH_2]$ (between cells) be caused by variation in C. For convenience of handling we may write o' for $\frac{-do}{dt}$ at a given locus. Then from (12) and (5)

$$o' = k_o P - k_o P_c = k_3 P_c [AH_2]$$

[5] The conclusion of LUND and MOORMAN (**19**) that the potentials of twelve frog skins bore no obvious relation to their rate of oxygen consumption offers no conflict to this view, if only upon statistical grounds. In fact their data suggest that for a large number of skins such a relation might appear.

or

$$P_c = \frac{k_o P}{k_o + k_3[AH_2]}$$

Substituting into (5) we obtain

$$o' = \frac{k_o P k_3[AH_2]}{k_o + k_3[AH_2]}$$

At any fixed and finite value of P, $[AH_2]$ will be determined by C, and

$$\lim_{C \to 0} o' = 0 \qquad \text{and} \qquad \lim_{C \to \infty} o' = k_o P$$

From (12)

$$P_c = \frac{k_o P - o'}{k_o}$$

and

$$r' = \frac{k_o P - o'_1}{k_o P - o'_2}$$

where the subscripts 1 and 2 designate respectively the positive and negative loci as measured externally. Then

$$\lim_{C \to 0} r' = 1 \qquad \text{and} \qquad \lim_{C \to \infty} r' = 1$$

For $0 < P < \infty$, $o'_1 < o'_2$ (equation 15), hence r′ varies for fixed P and changing C in the same way as for fixed C and changing P (fig. 2), and E_p rises from 0, passes through a maximum, and again descends to 0 as C increases.

The rate of oxygen consumption of the cell will increase with C. Under these conditions, and for the ascending limb of the E_p *vs.* C curve, the cell having the higher oxygen consumption will possess the larger E_p, and this will be true for all values of P between 0 and ∞. Thus the net electropositivity of the apex of polar tissues over the oppositely oriented basal regions is susceptible of explanation.

This relation carries the implication that the state of nutrition of a tissue determines the magnitude of its measured E.M.F., for it is well known that the nutritional state affects the rate of oxygen uptake. Direct experimental proof has recently been presented by LUND (**18**), who showed that skins from frogs fed on liver gave potentials 83 per cent. higher (and oxygen consumption 28 per cent. greater) than those from starved animals. This is supported by observations that captivity results in lowered potential (**5**, p. 362; **8**, p. 381). The clearest indication of a connection between magnitude of potential and concentration of oxidizable material exists in the phenomenon of "rebound" or "overshooting," a temporary increase in P.D. when P is changed from a low to a high value (**15, 8, 22**). The corresponding over-

shooting of oxygen consumption rate is of general occurrence and is evidently due to accumulation of AH_2.

The "apical" cell will also show a relatively greater increase in E_p with P. For simplicity let the E.M.F. represent the algebraic sum of the oppositely oriented polarity potentials of an apical and a basal cell. Then

$$E = \Sigma E_p = \frac{-RT}{2F} \ln \frac{r'_a}{r'_b}$$

The subscripts a and b respectively designate the apical and basal cells. The difference between two loci is determined by the constant k_1 and this difference is the same for the basal as for the apical cell. The apical cell is assumed to have the higher value of C. The variation of $\frac{r'_a}{r'_b}$ with P is plotted in figure 2, using the values of the constants given in table I. $\frac{r'_a}{r'_b}$

TABLE I

CONSTANTS OF THE CURVES IN FIGURE 2

	2A: $(k_1)_1 = 3$, $(k_1)_2 = 5$		2B: $(k_1)_1 = 5$, $(k_1)_2 = 10$	
	APICAL CELL (r'_a)	BASAL CELL (r'_b)	APICAL CELL (r'_a)	BASAL CELL (r'_b)
C	2	1	2	1
a_1	7	4	10.1	5.1
b_1	5	2	9.9	4.9
a_2	11	6	20.1	10.1
b_2	9	4	19.9	9.9

increases from 1 at P = 0, passes through a maximum, and returns to 1 at P = indefinitely large. The E.M.F. would increase from 0, pass through the maximum, and again return to 0. The curve is similar in form to r′ *vs.* P and the position of the maximum lies slightly beyond that for the r'_a curve. The rate of oxygen consumption of the apical cell in both cases is greater than that for the basal cell (calculated by equation 15 below). For the ascending limbs of the curves, the system satisfies the observed dependence of apico-basal polarity upon P.

Two cases are theoretically possible for the ascending limb. In figure 2A the apical cell is everywhere positive to the basal save at P = 0 and P = ∞. When the value of the ratio $\frac{k_2}{k_3}$ is small compared with the value of $\frac{k_1 C}{k_o}$, however, the tissue will show an inverted polarity at low values of the oxygen

pressure, as appears in figure 2B. Reversal of tissue polarity under these conditions is due to the fact that E_p increases more rapidly in the vicinity of $P = 0$ for the basal cell than for the apical.

Velocity of cell oxidation and oxygen pressure

The treatment given above yields a theoretical equation linking the velocity of cell oxidation to the oxygen pressure of the medium.[6] The rate of oxygen uptake at a locus is expressed by equation (7)

$$\frac{-do}{dt} = \frac{k_1 C k_3 P_c}{k_2 + k_3 P_c}$$

Upon substituting the value of P_c from (13) we obtain

$$\frac{-do}{dt} = \frac{k_1 C k_3 (P - a + \sqrt{a^2 + P^2 - 2Pb})}{2k_2 + k_3 (P - a + \sqrt{a^2 + P^2 - 2Pb})}$$

Dividing the numerator and denominator by k_3 and performing the substitution $\frac{2k_2}{k_3} = a - b$, gives

$$\frac{-do}{dt} = \frac{k_1 C (P - a + \sqrt{a^2 + P^2 - 2Pb})}{(P - b + \sqrt{a^2 + P^2 - 2Pb})} \qquad (15)$$

Equation (15) is plotted against P in figure 3 for six sets of values of the constants. $\frac{-do}{dt} = 0$ at $P = 0$; it increases rapidly at first, then more slowly, and approaches $k_1 C$ as a limit as P becomes indefinitely large.

The measured respiratory rate of a tissue or cell will be the sum of the $\frac{-do}{dt}$'s for all the loci. For simplicity we may write[7]

$$O' = \Sigma \frac{-do}{dt}$$

The characteristics of the curve O' *vs.* P will be similar to those of the individual $\frac{-do}{dt}$ curves, and the curve will approach $\Sigma k_1 C$ as a limit as P increases. This may be seen in figure 3, where curve (1) is obtained by adding together the points on curves (2), (3), and (4). The summation curve will follow the course of an equation identical in form with equation (15), or

$$O' = \frac{\Sigma k_1 C (P - a' + \sqrt{a'^2 + P^2 - 2Pb'})}{P - b' + \sqrt{a'^2 + P^2 - 2Pb'}} \qquad (16)$$

[6] For a recent review see TANG (24).

[7] O' is introduced to avoid possible confusion of the more generally used A with the same symbol for the oxidant of the electromotively active material. O'_m corresponds to the function A/A_o.

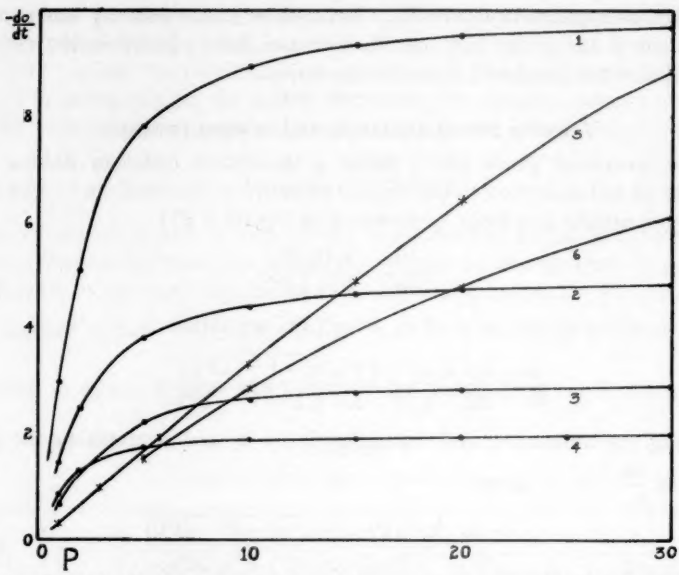

FIG. 3. Rate of oxygen consumption *vs.* oxygen pressure for the following values of the constants: (2) $k_1C=5$, $a=3$, $b=1$; (3) $k_1C=3$, $a=4$, $b=2$; (4) $k_1C=2$, $a=2$, $b=1$; (1), the summation curve of (2), (3), and (4) is fitted by the same general equation; values of the constants are $\Sigma k_1C=10$, $a'=2.98$, $b'=1.167$. (5) shows a nearly linear relation between consumption and pressure, $k_1C=10$, $a=30$, $b=29$. (6) $k_1C=10$, $a=30$, $b=5$.

a′ and b′ will lie within the extremes of the values of a and b respectively for the different loci. No general expression has been obtainable for determining their value.

Σk_1C may be eliminated in the following manner:

$$\text{Let } 0'_m = \frac{0' \text{ at P}}{0' \text{ at its maximum value}} = \frac{0' \text{ at P}}{\Sigma k_1 C}.$$

Then,

$$0'_m = \frac{P - a' + \sqrt{a'^2 + P^2 - 2Pb'}}{P - b' + \sqrt{a'^2 + P^2 - 2Pb'}} \qquad (17)$$

The behavior of 0_m *vs.* P for different values of a′ and b′ may be inferred from figures 3 and 4. The larger the value of a′ the more slowly does the curve approach its asymptote. For a given value of a′ the curve approaches its asymptote rapidly when $a'-b'$ is small, slowly when $a'-b'$ is large. With a′ and b′ very large and $a'-b'$ very small, the rate of oxygen consumption will appear linear over even a considerable range of P. In describing

experimental results of the linear type equation (16) would be employed, since the maximum rate of oxygen consumption is not determined.

Equation (17) has been applied to the majority of the data to be found in the literature, and in most cases yields a satisfactory fit. Space limitations prevent complete analysis of all available data; enough only is presented to illustrate the advantages and weaknesses of the equation as a description of experimental results.

Six sets of data are plotted in figure 4. The curves represent the course

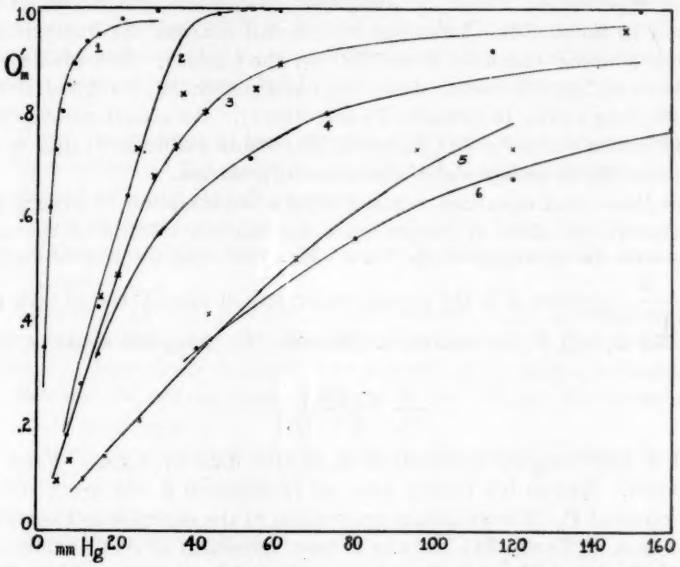

FIG. 4. Rate of oxygen consumption *vs.* oxygen pressure for six sets of published data: (1) SHOUP (23) luminous bacteria, $a' = 4$, $b' = 2.7$; (2) TANG and GERARD (25), fertilized *Arbacia* eggs, $a' = 35.4$, $b' = 35.26$; (3) LUND (13), *Planaria agilis*, $a' = 35.5$, $b' = 27.5$; (4) MEYERHOF (GERARD, 9, p. 267), NO_3 bacteria, $a' = 37.2$, $b' = 8.41$; (5) AMBERSON, MAYERSON, and SCOTT (1), *Homarus*, $a' = 113$, $b' = 90$; (6) THUNBERG (26), *Limax*, $a' = 93.6$, $b' = 17$. The curves follow the calculated values; observed values indicated by crosses or dots.

of equation (17); experimental points appear as crosses or dots. An arbitrary maximum was estimated for curve (5). For curves (2) and (6) the correspondence of observed and calculated values is nearly complete. SHOUP'S (23) results for luminous bacteria, curve (1), reach the maximum somewhat earlier than the calculated curve. Curves (3) and (5) show good correspondence except for the scattering of the experimental points. An incomplete fit is afforded the data of MEYERHOF on NO_3 bacteria (table in 9,

p. 267), shown in curve (4). Calculated and observed values correspond almost exactly until P reaches about 80 mm. Hg, beyond which the observed points deviate, reaching $O_m = 1$ in advance of the theoretical curve. Similarly data by TANG on unfertilized *Arbacia* eggs (**9**, p. 266) can be accurately fitted save for the upper three values. Determination of the point of 100 per cent. respiration is not always easy, and a moderate error may considerably alter the shape of the resulting O_m *vs.* P curve.

The equation will not follow data which show an upward concavity when plotted as above, *e.g.*, those of AMBERSON, MAYERSON, and SCOTT (**1**) for *Nereis*. In the modified form, however, it will describe the linear relation between O′ and P found by HALL (**10**) for the toadfish. Reasonable correspondence is found for other data (scup and puffer, **10**; fertilized *Arbacia* eggs, **25**; frog's skin, **15**; termite, **7**; and others). An almost point to point fit is given the curve for NO_2 bacteria, MEYERHOF (table in **9**) and to data by BODINE (**4**) on embryos of *Melanoplus differentialis*.

The theoretical equations relating oxygen consumption to oxygen pressure through the effect of oxygen upon the reaction velocity of the oxidative process are summarized by TANG (**24**). All take the hyperbolic form $A = \frac{P}{K_1 + K_2 P}$ where A is the oxygen consumption rate, P the oxygen pressure, and K_1 and K_2 are constants. GERARD (**9**), using the equation in the form

$$\frac{A}{A_0} = \frac{[O_2]}{K + [O_2]}$$

found K only roughly constant, if at all, for data by SHOUP, TANG, and MEYERHOF. Except for TANG's data the variation in K was greatest in the lower range of P. The graphical application of the equation to twenty-four sets of data by TANG (**24**) likewise is least successful at small values of P. (A scale for the ordinate in TANG's figures would make it possible to judge how well the equation really fits.)

In the "hyperbolic" equation P is interpreted to be the oxygen pressure of the medium, although upon the underlying assumptions it must be the intracellular oxygen pressure. The equation is identical with (7) above, and is valid only when P_c/P approaches 1. This will occur as $P \to 0$ and as $P \to \infty$; it will be true for all values of P *only* for cells in which $\frac{a+b}{2}$ is very small. It would be expected, therefore, that for small values of P the hyperbolic equation would show a poor fit for numerous experimental data, as GERARD and TANG apparently have found.

The estimation of the relative applicability of similar mathematical formulas to experimental results is uncertain. Nevertheless, because of its competency to follow experimental values in the lower range of P where

the hyperbolic equation tends to deviate, and because of the somewhat sounder theoretical foundation, the writer is of the opinion that the equation developed in this paper will prove more generally valid.

Finally it may be remarked that the derivation of a valid oxygen consumption: oxygen pressure relation upon the basis of the formulation of the reacting system for E.M.F. fulfills a necessary condition for the oxidation-reduction theory of bioelectric currents.

Summary

A quantitative connection is derived between the velocity of respiration and inherent cellular E.M.F. upon the basis of the amended steps in the oxidative reaction proposed by LUND. The system shows the following properties:

1. Direct dependence of the potential at a locus upon oxygen pressure.
2. Dependence of the electrical polarity of a cell (difference between two or more loci) upon oxygen pressure.
3. Direct dependence of the magnitude of cell polarity upon the concentration of the oxidizable material, *i.e.*, upon rate of oxygen uptake at constant oxygen pressure.
4. Relatively greater increase in cell polarity with increased oxygen pressure for the cell possessing the higher concentration of the oxidizable material, *i.e.*, dependence of apico-basal polarity upon oxygen pressure.
5. Reversal of cell or tissue polarity at low oxygen pressures under appropriate conditions.

An oxygen consumption-oxygen pressure equation is obtained which satisfactorily describes existing data.

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PHYSIOLOGICAL PROBLEMS CONNECTED WITH THE USE OF SODIUM CHLORATE IN WEED CONTROL

A. S. Crafts

Introduction

The use of sodium chlorate for controlling weeds has become a general agricultural practice within the past decade. The attendant experimental work has produced a voluminous literature and has brought into prominence many problems of a purely physiological nature. Since progress in the experimental work on chlorate apparently depends upon properly comprehending the mechanics of its distribution and absorption, work on these phases would have an immediate value. This paper presents several problems for consideration by plant physiologists and persons experimenting with weed control and discusses them in the light of our present knowledge.

Åslander (**1**) reported in 1926 that chlorates would kill plants by absorption from the soil, and in 1928 (**2**) he discussed critically the problem of weed control by this method. Meanwhile, however, the use of sodium chlorate solution as a spray (**11, 16**) claimed the attention of weed workers; and in the popularity of this method, Åslander's results were apparently overlooked. Most workers showed by their recommendations that they pictured the toxic action as taking effect through the plant after absorption of the chlorate by the leaves. Stickers, spreaders, various pressures, types of nozzles, and volume-concentration relationships were studied as they affected coverage of the foliage. Control of the hydrogen ion concentration of the spray solution, the inclusion of hygroscopic agents, and spraying methods in general were considered in attempts to improve the technique and avoid the erratic results so often encountered in the field.

Of twenty-nine publications on the use of chlorates that had appeared by the end of 1931, only three (**1, 2, 17**) emphasized the possibilities of the soil-application method. In 1931, Loomis, Bissey, and Smith, pointing out the advantages of this method, suggested that the action of sodium chlorate, as used in regions of summer rains, resulted largely from root absorption of chemical leached into the soil. Since then Muenscher (**20**) has pointed out the practical nature of the soil method, while Loomis and others (**18**) have given further experimental evidence of its feasibility.

Meanwhile there had been developed in California a method for treating certain deep-rooted perennials with acid arsenical spray (**4, 5, 8, 15**). Its success depends upon translocation of the toxicant within the vascular system of the plant. The question naturally arises, will this same mechanism

carry chlorate in the plant and, if so, to what extent does it explain the results obtained in the common use of chlorates?

As the writer has recently reported (**6**), chlorate may be moved through plants and may kill the roots to considerable depths as a result of leaf absorption and translocation within the xylem. The effects of a given treatment are limited by the same factors that govern penetration and root killing by the acid arsenical. When conditions are right, any application of chlorate solution to the foliage will result in some absorption and downward movement within the plant. The extent of injury will depend upon how nearly the requirements for successful treatment by this method are met.

Besides these two distinct mechanisms available for use in treating deep-rooted weeds, there are several interesting physiological responses by the plant to both lethal and sublethal doses of chlorate. The various effects that chlorates may have on plants will therefore be listed and discussed. Wherever possible, their relation to weed-control practice will be pointed out.

Physiological action of chlorates

There are, apparently, four fundamentally different ways in which chlorates may affect plants:

1. When a chlorate solution is applied to leaves, ions of the salt diffuse through the cuticle and come into contact with the protoplasm of living cells (**18, 5**). Although the coefficient of permeability of plant cells to chlorate ions is low (**23**), when they attain a lethal concentration they enter the plant cell, causing injury and eventually death. The exact mechanism by which this result is accomplished is not known. It has been suggested that the high oxidizing potential of sodium chlorate (**22**), the presence of pentavalent chlorine (**22**), and the complete oxidation of respiratory chromogens (**12**) may be involved; but no experimental evidence has been presented to support these ideas. Contrary to popular notion (**14, 22, 23, 24, 26**), sunlight or ultraviolet light is not essential to this process, which will take place in the dark (**17**); and open stomata (**19**) are not necessary, the chemical being fully toxic when applied to the ventral surfaces of hypostomatic leaves (**5, 18**). The solutions commonly applied as sprays in no way compare in concentration with those generally used in physiological experiments (**23**). They usually become saturated and therefore strongly plasmolytic soon after application, a steep gradient in concentration developing across the cuticle layers.

2. When chlorate ions exist in the plant in sublethal concentration, there is a typical response, characterized by a chlorotic, stunted condition of any growth that occurs, a reduction of the starch reserves (**16**), decreased catalase activity (**21**), and increased susceptibility to frost injury (**16**). These

symptoms may precede death of the tissues if conditions favor the continued uptake and accumulation of chlorate. Where additional chlorate is not available, the plant may recover and show no permanent effects.

3. Under certain limited conditions, a concentrated chlorate solution applied to the leaves of plants may penetrate the cuticle, plasmolyze and kill the mesophyll tissues, enter the xylem, and be carried down into the roots (7). The conditions essential to this action have been discussed in connection with acid arsenical sprays (4, 5, 8, 15). Experiments to be described indicate that the same response may occur with chlorates and will result in a rapid, deep killing of the root system.

4. Chlorates present in solution in the soil may be absorbed by roots, killing all parts in which they accumulate to a lethal concentration.

Although it has been intimated that chlorates may penetrate the leaves and be translocated through the phloem (11, 22), this possibility seems rather remote in view of the prevailing concepts of phloem tissues (3, 9). Movement of chlorate through a system so dependent upon the functioning of living cells is hard to imagine.

Under different conditions of treatment the four effects listed may occur in almost any combination, singly or together. Rarely in the field are less than two concerned; and often all four may be in evidence during the year. Naturally, therefore, results of plot tests have been difficult to interpret, and recommendations from different localities have been inconsistent and confusing.

Before discussing these four responses of plants to chlorate in greater detail, it would be well to consider briefly the inherent differences in plants with respect to injury in general and also with respect to injury by chlorates.

Many plants are easily killed. When cut off at the ground level, they fail to recover and die with no further treatment. Other plants will resprout from the stump or crown, and still others may regenerate from stem, rhizome, or root tissue and are eradicated only when all vestiges of the root are killed or removed from a suitable environment. In treating a variety of plants with a toxic substance like chlorate, one is sure to find differences in the degree of control; and unless he has previously determined their response to mechanical injury he cannot accurately interpret his results.

Furthermore, plants vary widely in their susceptibility to injury by chlorates. Although this fact has been pointed out many times (6, 10, 13, 28, 29), it will bear repeating because it is a striking phenomenon, vitally involved in the determination of dosages. Until one has determined in an empirical way the susceptibility of a particular species, he cannot well prescribe the dosage necessary for its control in the field.

Killing of plant tissue by chlorate

It has been pointed out that whenever chlorate ions come in contact with plant cells in sufficient concentration, the tissue is killed. Though the mechanics of this killing process form an interesting field for speculation, the critical point, so far as weed control is concerned, is that only the presence of chlorate in lethal concentration is necessary for the death of the cells. Our principal problem is distribution of the toxicant. For the desired results, it must come into contact with the vital tissues.

Obviously, therefore, the technique of application must be related to the plant concerned. If the plant is easily killed, a thorough spraying of the foliage is sufficient. If it resprouts weakly, spraying of the regrowth may effect a kill. On the other hand, if the plant regenerates strongly from the roots, killing of the tops is a small part of the problem, and spraying is effective only as it may lead to leaf absorption and rapid killing by xylem transport as described in (3), page 701, or as it distributes the chemical for leaching and absorption from the soil. Apparently the killing of the cells by chlorate is largely independent of the environment and is a matter only of concentration and cell activity.

Physiological responses of plant cells to chlorate

The effects of chlorate upon plants have considerable interest for workers in plant physiology. Concentrations in the tissues near or below the lethal point cause reactions that are apparently peculiar to chlorate alone. LATSHAW and ZAHNLEY (**16**) pointed out the great reduction in starch reserves of roots after chlorate treatments. The writer has observed the same phenomenon not only in sprayed plants but in those which have absorbed chlorate from the soil. It has been seen in stems of plants containing a sublethal concentration and is undoubtedly present in all tops showing the stunted chlorotic growth that commonly follows dusting with Atlacide, or soil applications. Lowered catalase activity (**21**) and susceptibility to frost injury (**16**) accompany this condition; and, taken together, these responses indicate a lowered vitality in the presence of chlorate ions.

As has been mentioned, when conditions favor continued absorption of chlorate, this condition of lowered vitality is accentuated, so that the tissues finally die. With lowering chlorate concentration, however, they may recover and show no permanent injury. From the standpoint of weed control this response of plants to chlorates is of minor importance, being useful only as an indicator of the presence of the chemical.

Killing of plants with chlorate following leaf absorption and transport to the roots through the xylem

Having already been described, the action of this mechanism (**4, 5, 8, 15**) requires no further comment. That it will respond to chlorate sprays has

been demonstrated (**6**). Its possibilities in weed control, however, are of interest; and its relation to results obtained by present methods warrants comment.

LOOMIS and others (**18**) have shown that chlorate ions will enter and move through the xylem of plants. Their results are not surprising, for any solution of molecularly dissolved substance that does not undergo chemical change within the xylem will displace the xylem sap and follow the transpiration stream wherever the gradients in pressure may cause it to flow. The critical point is, will chlorates kill the leaf cells and, rendering them permeable, penetrate the xylem and be carried deep into the roots, causing their death? Recent developments (**4**) indicate that this method has practical possibilities, after all, and that chlorates might find a logical use in certain cases.

Although some evidence has been presented previously (**6**) and although this paper does not aim primarily to submit extended experimental results,

TABLE I

EFFECT OF TIME OF DAY, ACIDITY, AND CONCENTRATION UPON THE ROOT KILLING OF MORNING-GLORY PLANTS BY SODIUM CHLORATE

PLOT NO.	$NaClO_3$ PER SQ. ROD	H_2SO_4 PER SQ. ROD	TIME OF APPLICATION	PLANTS RESPROUTING OCTOBER 8, 1931
	lb.	*lb.*	P.M. Aug. 20, 1931	%
1	1	.....	4:00	90
2	1	.....	7:00	90
3	2	.....	4:30	90
4	2	.....	7:30	40
5	3	.....	5:00	80
6	3	.....	8:00	10
			P.M. Aug. 21, 1931	
7	1	.....	4:00	90
8	1	.....	7:00	90
9	2	.....	4:30	90
10	2	.....	7:30	30
11	3	.....	5:00	80
12	3	.....	8:00	5
			P.M. Aug. 22, 1931	
13	1	1.25	4:00	10
14	1	1.25	7:00	10
15	2	1.25	4:30	40
16	2	1.25	7:30	10
17	3	1.25	5:00	10
18	3	1.25	8:00	5

the data on a few trials will be given to show the nature, possibilities, and limits of this method.

Table I presents the results of spraying a series of plots with sodium chlorate solutions in August, 1931. These applications were made during a period when the daytime temperatures reached 100° F. Though temperatures were somewhat lower toward evening, the relative humidity was low, and all sprays applied before sundown dried very rapidly on the leaves. The plots were in a young orchard that had been irrigated and disked late in June. A dense growth of morning-glory had matured and had lowered the soil moisture to a point approaching the permanent wilting percentage at the time the sprays were applied. Each plot was approximately 1 square rod in area, and the chemical was applied in 3 gallons of water. The foliage on all plots was killed within 48 hours.

As no rain fell between August 20 and October 8, 1931, the results at the latter date represent the action of chlorate on and through the plants. The plants on the plots showing low percentages of resprouting were killed to a depth of 3 feet or more. Glancing over the data, one observes that the more concentrated sprays were the more effective, that delaying the application until after sundown materially improved the results, and that sulphuric acid markedly increased the efficiency of the method.

Table II presents the results of some plot tests with sodium chlorate applied on September 28, 1931. Plots 4 to 6 and 10 to 12 in this series had been recently irrigated. The temperature at this date was lower and the relative humidity higher, so that penetration during the day was considerably better than in August. These results again show the effects of concentration of the solution and the value of adding acid to increase the rate of penetration. In addition they show the effect of soil moisture upon the action of the mechanism. Even though transpiration was high at this season, the plants in the moist soil did not have the high water deficit of the others, and distribution of the toxicant in the roots was less complete.

These experimental results are typical of a good many obtained during the work with chlorates. They indicate that this method has certain possibilities in areas where the plants deplete soil moisture rather thoroughly. In regions of frequent summer rains it would have no value.

In considering the practical possibilities of this method, one must note several points. First, it would probably never be more effective than the acid arsenical, and the chemicals will necessarily cost from three to five times as much. Second, the acid cannot be applied along with the chlorate, for the combination forms a strong oxidizing mixture that will ruin any machinery; it would have to be applied as a separate spray following the chlorate application. Finally, applications under the proper conditions would involve the fire hazards attending the use of chlorates.

TABLE II

EFFECTS OF APPLICATION RATE, SOIL MOISTURE, AND ACID UPON CHLORATE KILLING OF MORNING-GLORY

PLOT NO.	DATE IRRIGATED	SPRAYED SEPT. 28	$NaClO_3$ PER SQ. ROD	H_2SO_4 PER SQ. ROD	RESPROUTING NOV. 23, 1931
			lb.	*lb.*	%
1	Aug. 15	4:20 P.M.	1	.....	20
2	Aug. 15	5:20	2	.....	15
3	Aug. 15	3:45	3	.....	5
4	Aug. 15	4:10	1	.....	45
5	and	5:10	2	.....	35
6	Sept. 24	3:15	3	.....	40
7	Aug. 15	4:40	1	1.25	1
8	Aug. 15	5:00	2	1.25	0
9	Aug. 15	5:40	3	1.25	0
10	Aug. 15	4:30	1	1.25	25
11	and	4:50	2	1.25	25
12	Sept. 24	5:30	3	1.25	25

On the other hand, with due caution this method might be used in pasture areas where the acid arsenical would present a poison hazard. Also, where conditions are optimum for this type of spray, the experimental results indicate that the chlorate dosage may be materially reduced. Furthermore, the application of sulphuric acid in a dosage providing a weight of the concentrated acid equal to that of the sodium chlorate will liberate chloric acid and reduce the chlorate ions reaching the soil so that little or no residual effect will be found. Sulphuric acid is best applied in a concentration of about 5 per cent. by weight or approximately one normal. Even this is corrosive to machinery and requires special acid-resistant equipment. Such equipment is available for using sulphuric acid as a spray on mustard in cereals.

KILLING OF PLANTS WITH CHLORATES BY ABSORPTION FROM THE SOIL

It is apparent that treatment of deep-rooted weeds through the soil is a logical method. The regenerative organs themselves are directly affected by the chemical; with proper dosage and distribution there is little possibility of failure. The method is not subject to those diverse and difficultly controlled factors to which the foliar organs are exposed.

In the problem of dosage, species susceptibility and the effect of soil type on chlorate concentration are vital factors. The problem of species

susceptibility has been mentioned. Variations are extremely wide. TIMSON (25) describes a parasitic plant called "witch weed" that requires a maximum of only 80 gallons of spray containing 12 pounds of sodium chlorate per acre for control of a solid infestation. At the other extreme, chlorates have often been applied to hoary cress and other of the less susceptible species at rates of 6 and 8 pounds per square rod with only partial kills. Though these latter results may be due in part to improper methods, more susceptible species treated under identical conditions are often completely eradicated.

At present the only hope for determining the dosage to use on a particular species in the field is an empirical test under local conditions. Though the operator may be guided somewhat by results described in the literature, soil and climatic factors so affect the growth of plants that local experience is usually required.

The influence of soil type upon chlorate toxicity, having been introduced in another place (7), will be only touched upon here. Though toxicities may vary as widely as five times between soils of different types (7), no generalization can be offered now that will aid in their determination. Again empirical testing is indicated as the most promising method for studying this effect. It seems at present that, within a soil series, toxicity will run higher in the coarser grades. Among series, recent alluvial soils exhibit the lowest toxicity; old weathered soils the highest. Much more work is required, however, before these statements can be proved. The results of such testing at this station will appear as the work is continued.

The fixing of chlorate in a form available to plants is another soil property to be considered. As experiments have shown (7), certain soils are able to hold chlorates so that they do not move freely in the soil solution. In general the soils showing the lowest toxicity have the strongest fixing power, and in these the proper vertical distribution of chlorate within the soil is a problem. Under the conditions at Davis, morning-glory must be killed to a depth of at least 4 feet or it will resprout and survive. A lethal concentration of chlorate, therefore, must be provided throughout the top 4 feet of soil if the treatment is to be a success. In a Yolo silt loam, leaching experiments showed (6) that about 6 inches of water were required when the chlorate was applied to moist plots, and from 8 to 12 inches when dry plots were treated. In a heavier soil these requirements might well be doubled. Where the moisture came as rainfall with opportunity for evaporation between storms, even more water would be needed. This one factor probably explains many of the failures attending the use of chlorates in the arid regions of the west.

Soil moisture enters the problem again in the matter of chlorate decomposition. Where rains keep the soil wet during the hot summer season,

residual effects from chlorate treatments are of little importance. Where the top soil dries out during the summer, chlorate remains intact and may persist for three years (**6**) or more. Under these conditions leaching with irrigation water is the easiest method for ridding the land of chlorate (**6, 7**). Where irrigation is not available, chlorates should be used with considerable discretion on heavy soils.

The proper season for chlorate application has been much debated. Many publications recommend the blossoming stage of the plants, which usually occurs in early summer. Sometimes the same recommendation will state that the plants should be mature or *fully* mature for successful treatment. In central California morning-glory comes into blossom in April and May. On unirrigated land it reaches maturity, as denoted by ripening of seed and cessation of terminal growth, in June and July. On irrigated land it may not mature until October. Obviously, therefore, these recommendations cannot be followed in any one treatment.

HULBERT, BRISTOL, and BENJAMIN found in Idaho (**13**) that treatments from May to August were equally effective by the following summer. WILLARD (**27**), and SAMPSON and PARKER (**24**) treated successfully during the spring; ÅSLANDER (**1, 2**), MUENSCHER (**20**), and the writer (**6**) have had excellent results from winter treatments. Stage of growth therefore would appear to have little effect in this response. Careful analysis, however, indicates that this is not altogether true. LOOMIS and others state: ". . . sodium chlorate dissolved in the soil water readily penetrates and kills the roots and rhizomes of either active or dormant plants" (**18**). Although this is undoubtedly true, absorption of chlorate by dormant roots is apparently slower than by active ones. The writer has noted many cases where plants have survived fall and winter applications only to weaken and die in the early summer. Spring and early summer treatments through the soil have been generally more successful than summer and fall applications except as the latter have provided a lethal concentration in the soil for absorption during the following spring. Midsummer applications have given the greatest success only in regions of summer rainfall where the chlorate is very soon made available for absorption through the roots.

The plant root goes through three definite stages during the year. In the spring and early summer it is in a vegetative state characterized by rapidity of growth, absorption of water and mineral nutrients, and depletion of organic food reserves. During the summer and fall it replenishes its food reserves; growth and absorption become slower. During the winter, growth and absorption are at a minimum, and organic foods are only slowly changed. From the standpoint of its physiology, the root should be most susceptible to chlorate injury during its vegetative stage in the spring. The discussion just presented indicates that this is probably true.

At least two important factors are involved in this increased susceptibility during the spring. Rapid absorption of water and mineral nutrients by the roots should favor absorption and accumulation of chlorate within the plant, and the low level of food reserves should make the roots vulnerable. On the other hand, the gradual killing of plants in the spring following fall and winter applications takes place generally where a given dosage has been leached to a considerable depth and the actual chlorate concentration at any point is low. With the depletion of soil moisture, the chlorate concentration increases generally throughout the soil mass. In addition, because of the water moving into the plant roots and the selective rejection of chlorate resulting from the low coefficient of permeability of protoplasm for these ions, the chemical must concentrate at the surface of the root. As the ions concentrate to the lethal point the roots become injured, increasing in permeability, and then chlorate enters the plant in much greater amounts than before. The result is the complete breakdown and death so often noted as the soil moisture runs low. The effective concentration at the absorbing surfaces must be much higher than if the soil moisture were maintained by irrigation or rainfall. That this factor of concentration within the soil is important is indicated by the number of recommendations that warn against irrigating after chlorate applications. The writer has used irrigation water (**6**) for attaining a vertical distribution of chlorate in the soil with considerable success. Apparently it is harmful only where it maintains a high moisture content that effectively dilutes the chlorate, or where it leaches the chlorate beyond the region of absorbing roots.

Discussion and summary

Chlorate, as a plant poison, seems to have unique properties. It acts slowly (**18**) compared with the heavy metals, seeming to enter the plant in low concentration, and gradually accumulates if the source of supply is maintained. Though arsenic, when present in the soil, affects principally the absorbing organs and has little primary effect upon the tops, chlorate apparently affects the whole plant at the same time and in essentially the same way. Seedlings that have germinated in soils containing large amounts of arsenic are often found to have their roots so injured that water is absorbed through dead tissues as through a wick. Plants in soils containing chlorates in just as injurious quantities have strong root systems. Here the plants may exhibit considerable growth and then dry up and die completely. Very high concentrations of chlorate in the soil will kill roots just as does arsenic.

The writer is reminded of some early experiments with arsenic and chlorates on morning-glory shoots. Excised shoots placed in dilute solutions of these two chemicals react characteristically. The young tender tips of the shoots in arsenic solutions turn black and droop within 16 hours, and

the older leaves die later. With the chlorate-treated shoots, the older leaves die first; the tips turn chlorotic but do not die, and many actually grow for several days, elongating to the extent of 10 to 20 centimeters. Apparently, arsenic is primarily a protoplasm poison and kills as it goes, entering young and old tissues alike. Though chlorate will also kill by contact when present in high concentrations, it seems to enter the plant more slowly when absorbed from the soil or when applied in solutions of low concentration and allowed to act by accumulation in the tissues. The plant dies under these circumstances, not because the protoplasm has been killed by direct reaction with the toxicant, but rather because this material so disturbs the metabolic processes that the plant can no longer function normally. The symptoms are systemic in nature, and apparently assimilation and utilization of foods as well as other vital functions are affected.

The discussion presented shows that there can be no universally successful method for using chlorates. With the extreme variations in susceptibility of species and the number of factors affecting toxicity and absorption, methods must be adapted to the conditions of the treatment, the operator taking every possible advantage of the situation at hand. Though summer rains provide an ideal means for distributing the chemical in the soil, insuring the success of spring and summer applications in the humid regions, other methods must be used in the more arid parts of the west. Directions provided by chemical companies and experiment stations for the use of chlorates should be adapted to the locality in which the chemical is to be used.

The successful use of chlorate is obviously more difficult in arid regions. In California, three methods have been proposed (**6**): (1) fall spraying where rapid absorption and root killing are followed by leaching and absorption from the soil; (2) a straight soil treatment during the winter; (3) spring soil treatment followed by proper irrigation. All three methods, however, are subject to differences in growth conditions and soil type, and the first two depend upon rainfall. They must be used, therefore, with utmost care.

All soil applications should aim to provide a toxic concentration of chlorate throughout a proper depth of soil for absorption during the spring vegetative season. Plants left undisturbed in such soils accumulate chlorate as the moisture decreases and grow weaker with the advancing season. Hoeing or weed cutting will eliminate the few plants that struggle along and sometimes survive this treatment (**6**). This should not be done, however, until the plants are severely affected. At least one complete season must be allowed for success by the chlorate method, and provision must be made for destroying such seedlings as appear in succeeding years.

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PHYSICAL AND CHEMICAL PROPERTIES OF THE SOLUBLE POLYSACCHARIDES IN SWEET CORN

M. W. PARKER

(WITH ONE FIGURE)

Introduction

The endosperm of sweet corn, in contrast to that of most varieties of maize, contains a relatively high percentage of soluble polysaccharides, but very little is known concerning their physical and chemical properties. On the basis of the iodine color alone, CULPEPPER and MAGOON (**8**) concluded that these water soluble polysaccharides are composed of both dextrins and soluble starch.

This investigation deals with the isolation and purification of the water soluble polysaccharides from green and mature Hopeland sweet corn kernels, and with their physical and chemical properties. These properties were compared with those of alpha and beta amylose from mature Hopeland sweet corn starch. The chief purpose of this comparison was to determine whether the soluble polysaccharides are true dextrins produced by starch hydrolysis or whether they are synthesized amyloses that have not been organized into starch grains.

Investigation

EXTRACTION AND PURIFICATION OF SOLUBLE POLYSACCHARIDES

The green sweet corn was prepared for extraction of the soluble polysaccharides by splitting the kernels and scraping out the contents. The air dry mature corn was first soaked in 20 per cent. alcohol and the softened kernels were then ground through a Nixtamal mill. Enzymatic action was checked during the extraction by 1×10^{-4} N iodine (**18**).

These polysaccharides are soluble in water and in alcohol up to 30 per cent. by volume and are precipitated by alcohol above this concentration. Therefore 20 per cent. alcohol was used as the solvent during extraction and purification. Samples of the corn pulp were extracted twice with cold 20 per cent. alcohol. The first extraction was made soon after adding the alcohol while the second was made after shaking for one hour. The extracts were separated from the pulp by means of several thicknesses of cheesecloth. These extracts contained the soluble polysaccharides, starch, and various other substances. The starch was removed from the extracts by centrifuging and filtering. The soluble polysaccharides were then precipitated from the filtrate by increasing the alcoholic concentration to 60 per cent. by volume. The 60 per cent. alcohol was removed and the material was re-

dissolved in 20 per cent. alcohol. This solution was centrifuged and filtered in order to remove impurities. The process of dissolving in 20 per cent. alcohol and reprecipitating in 60 per cent. alcohol was repeated until no residue was deposited on centrifuging the 20 per cent. alcoholic solution and qualitative tests for proteins in the wash alcohol were negative. Final purification of the soluble polysaccharides was effected by dissolving in water, filtering, and subjecting the filtrate to electrodialysis. The apparatus (fig. 1) employed for electrodialysis is a modified form of the chamber used by

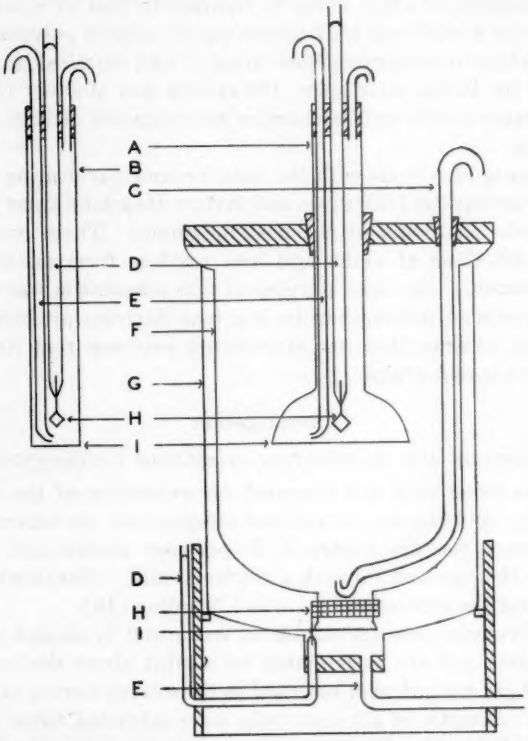

FIG. 1. Electrodialysis apparatus: *A*, improved negative electrode chamber; *B*, negative electrode chamber used until 1932; *C*, siphon for dialysis chamber; *D*, mercury connections for electrode; *E*, siphon for electrode chamber; *F*, dialysis chamber; *G*, positive electrode chamber; *H*, platinum electrodes; *I*, collodion membranes.

TAYLOR and IDDLES (**23**). The electrode chamber which was used for the purification of the samples taken in 1930 and 1931 is shown in figure 1 *B*; the electrode chamber which was adopted in 1932 is shown in figure 1 *A*. The new electrode chamber has a much larger membrane surface and allows

a larger volume of water to surround the electrode. This improvement hastened the separation and purification of the materials investigated. The membranes of the electrode chambers were of collodion supported on cheesecloth and were of such thickness that they allowed the free passage of inorganic ions while retaining the polysaccharides. A current of 250 volts d.c. was passed through the solutions until they were free of unbound inorganic ions. This point was detected by the conductivity of the water in the electrode chambers. The water in these chambers was changed frequently during the first 24 hours of a run; from then on once a day was sufficient.

The water soluble polysaccharides were divided into two major fractions by electrodialysis. One fraction migrated to the positive electrode and was deposited as a gelatinous mass around the positive membrane while the other fraction always remained in suspension during electrodialysis. These fractions were tentatively named alpha and beta respectively. The purification of the alpha and beta fractions of the soluble polysaccharides was readily effected by siphoning the supernatant beta fraction from the chamber. Water was again added to the alpha fraction and electrodialysis was continued until a clear separation had occurred. This process was repeated until the water above the alpha fraction was free of the beta fraction, as evidenced by no precipitate in 60 per cent. alcohol and no coloration with iodine. The purified alpha and beta fractions were precipitated in 70 per cent. alcohol, washed in absolute alcohol, and dried in a vacuum over sulphuric acid at room temperature. Although the beta fraction always remained in suspension during electrodialysis, a portion of it migrated toward the positive electrode. The properties of this portion were identical with those of the non-migrating portion.

Separation and purification of alpha and beta amylose from starch

The starch from air dry mature sweet corn which had been separated from the soluble polysaccharides by centrifuging was purified by washing in 20 and 80 per cent. alcohol. The starch was washed and centrifuged eleven times in 80 per cent. alcohol before it was considered pure.

It is well known that the starch grains must be completely ruptured before a separation of alpha and beta amylose can be made. In order to avoid the possibility of any change in the beta amylose arising from the use of chemical agents, the method of mechanical rupture as reported by Alsberg and Perry (**1**) and Taylor and Beckmann (**22**) was adopted. This method gave complete rupture of the starch grains after grinding in a ball mill for one week, as evidenced by the disappearance of the typical black cross on the starch grains when viewed between crossed Nicol prisms.

An 8 per cent. suspension of ground starch in water was subjected to electrodialysis. The alpha amylose migrated to the anode and the clear supernatant beta amylose was siphoned off and precipitated in 75 per cent. alcohol, washed in absolute alcohol, and dried in a vacuum at room temperature. The alpha amylose was repeatedly resuspended in water and electrodialized until free of beta amylose. This required 59 washings. The alpha amylose was then frozen and the frozen mass allowed to thaw as it was filtered in a Buchner funnel. Drying was completed in a vacuum desiccator over sulphuric acid at room temperature.

Physical and chemical methods

Color with iodine.—The iodine coloration of a solution was determined by adding 10 drops of N/100 iodine potassium iodide to 10 ml. of a 1 per cent. solution.

Viscosity.—The relative viscosity was determined with an Ostwald viscosimeter. The measurements were made on 5 ml. of a 1 per cent. solution at 25° C. ± 0.03. All of the determinations were made immediately after preparing the solutions. Each value for relative viscosity is the average of at least two samples and each sample was run through the viscosimeter ten times.

Optical rotation.—A Schmidt and Haensch half-shadow saccharimeter was used to determine the optical rotation. The readings were obtained in Ventzke degrees which were converted to angular rotation by the factor 0.34657. It was necessary to use dilute solutions of the beta soluble polysaccharide preparations because of their opalescence. In determining the optical rotation of samples which had been hydrolyzed, the original weight of the sample was used to calculate the specific rotation. The specific rotations reported for the unhydrolyzed samples are the average of two samples, and five to six readings were made on each sample. The specific rotations for the hydrolyzed materials are from one sample, on which five to six readings were made.

Electrical conductivity.—The apparatus employed for these determinations consisted of the usual equipment for such measurements and was set up as shown by Getman (9). A Leeds and Northrup student's potentiometer was used as a wheatstone bridge. The determinations were made on 1 per cent. solutions at 25° C. ± 0.03. Each conductivity reported is the average of two samples. The conductivity of each sample was determined nine or more times with the fixed resistance at three different points.

Flocculation by salts.—The samples of 10 ml. of 2 per cent. solutions were treated with half saturated ammonium sulphate, basic lead acetate, and 0.1 N iodine potassium iodide.

ASH.—The materials were ashed in platinum crucibles at dull redness in a muffle furnace. The ashings were continued until the crucibles were constant in weight after 30-minute heating intervals.

PHOSPHORUS.—All of the ashings for the phosphorus determinations were made by the wet ashing method following the procedure recommended by ZINZADZE (**25**). The ashings were performed on an electric sand bath at 165°–170° C., since ROE, IRISH, and BOYD (**20**) have shown that phosphoric acid is volatized above 200° C. The colorimetric method employed for the beta fractions is that of BELL and DOISY (**5**) as modified by BRIGGS (**6**) and improved by ROE, IRISH, and BOYD (**20**). The procedure for this determination has been reported by COCKEFAIR (**7**). The standards were prepared from potassium dihydrogen phosphate simultaneously with the unknown solutions. Since ROE, IRISH, and BOYD (**20**) have shown that the acidity has a marked influence on the color produced, it was necessary to add 1 ml. of concentrated sulphuric acid to the standards in order to make the acidity the same as that of the unknown solutions. The solutions were matched with a Bausch and Lomb Duboscq colorimeter, using 50 mm. cups.

The volumetric method employed for the alpha fractions is essentially that of PEMBERTON (**19**) as reported by MATHEWS (**16**). The ammonium phospho-molybdate was washed with water until free of acid, and then dissolved in N/10 sodium hydroxide. This solution was titrated with N/10 sulphuric acid.

FATTY ACIDS BEFORE AND AFTER HYDROLYSIS.—The samples which had been dried at 80° C. under 3 to 4 cm. pressure were extracted in a BAILEY-WALKER (**2**) extraction apparatus for 24 hours with redistilled anhydrous ethyl ether. The extract was evaporated and dried at 80° under 3 to 4 cm. pressure. Any residue remaining after correcting for the blank determination was considered unbound or extraneous fatty material.

The extracted samples were hydrolyzed according to the method of TAYLOR and NELSON (**24**) and filtered through fat-free filter paper. The residues were washed free of acid, and after drying the filters and the residues at 50° C., they were extracted with anhydrous ethyl ether. The weight of the ether extract was determined in the manner previously described.

REDUCTION OF FEHLING'S SOLUTION BEFORE AND AFTER HYDROLYSIS.—The reducing power of 50 ml. of 1 per cent. solutions was determined by the Munson and Walker gravimetric procedure (**17**). The reducing power of samples which had been hydrolyzed for 2.5 hours with 2.5 per cent. sulphuric acid was also determined by this method. The percentage of glucose formed on hydrolysis was calculated by using the factor 0.9, which allows for the increase in weight on hydrolysis.

MELTING POINT OF OSAZONES.—Samples which had been hydrolyzed were neutralized and treated with phenyl-hydrazine hydrochloride and sodium

acetate in a boiling water bath for 1 hour. The resulting osazones were recrystallized and their melting point determined by the capillary tube method. All of the melting points were corrected according to the formula of KOPP (**11**). The melting points reported are the averages of two or three determinations.

NITROGEN.—The nitrogen determinations were made by the usual Gunning method (**17**). Distillation was made into 0.1 N sulphuric acid and the excess acid was titrated with 0.1 N sodium hydroxide using the combination methylene blue-methyl red indicator (**10**).

Results

PROPERTIES OF BETA FRACTION OF SOLUBLE POLYSACCHARIDES PREPARED FROM SWEET CORN IN DIFFERENT YEARS

The beta fraction constitutes from 78 to 80 per cent. of the water soluble polysaccharides in sweet corn at the milk stage. The properties of this fraction of the soluble polysaccharides prepared from two different crops of sweet corn in the milk stage were practically identical. Both prepara-

TABLE I

PHYSICAL PROPERTIES OF BETA FRACTION OF SOLUBLE POLYSACCHARIDES PREPARED FROM TWO CROPS OF SWEET CORN IN THE MILK STAGE

YEAR	RELATIVE VISCOSITY AT 25° C. ± 0.03	SPECIFIC CONDUCTIVITY AT 25° C. ± 0.03 × 10^{-6}	SPECIFIC ROTATION AT 25° C.	SPECIFIC ROTATION OF HYDROLYZED SAMPLES AT 25° C.
1930	1.0715	9.40	195.8	56.3
1931	1.0750	3.39	195.2	58.7

TABLE II

CHEMICAL PROPERTIES OF BETA FRACTION OF SOLUBLE POLYSACCHARIDES PREPARED FROM TWO CROPS OF SWEET CORN IN THE MILK STAGE

YEAR	P_2O_5	CONVERSION TO GLUCOSE ON HYDROLYSIS	MELTING POINT OF OSAZONES FROM HYDROLYZED SAMPLES	TOTAL NITROGEN*
	%	%	° C.	%
1930	0.0079	95.22	208.8	0.05
1931	0.0013	97.96	205.9	0.01

* Single determination.

tions were white amorphous powders which were exceedingly hygroscopic. They were not flocculated by basic lead acetate, half saturated ammonium sulphate, or iodine potassium iodide. Neither of the beta preparations contained any weighable ash or any fatty material. Both preparations were non-reducing and exhibited the same purplish violet iodine color. The results of other physical and chemical measurements on these two preparations are shown in tables I and II. The material prepared in 1930 had a higher conductivity than the material prepared in 1931. This may be due to the fact that the 1930 preparation had more bound phosphorus and also more nitrogen. The nitrogen is probably due to impurities which had not been completely removed from the 1930 preparations.

Comparison of properties of beta fraction of soluble polysaccharides with those of beta amylose prepared from mature sweet corn starch

Preparations of the beta fraction of the soluble polysaccharides from corn in three stages of ripening were compared with one another and with beta amylose from mature sweet corn starch. The solutions of beta fractions of soluble polysaccharides were very opalescent and the iodine color was purplish violet, while the beta amylose solutions were water-clear and gave a pure blue iodine color. There was no ash or bound fatty material in any of the beta fractions or in beta amylose. The materials compared did not reduce Fehling's solution and they were not flocculated by half-saturated ammonium sulphate, basic lead acetate, or iodine potassium iodide. On the addition of a small amount of an electrolyte to the solutions, the beta amylose was flocculated by iodine potassium iodide as reported by

TABLE III

Comparison of physical properties of beta fraction of soluble polysaccharides and beta amylose from sweet corn starch

Material	Relative viscosity at 25° C. ± 0.03	Specific conductivity at 25° C. ± 0.03 $\times 10^{-6}$	Specific rotation at 25° C.	Specific rotation of hydrolyzed samples at 25° C.
Beta fraction of soluble polysaccharides				
Milk stage	1.0750	3.39	195.2	58.7
Dough stage	1.0723	3.35	195.8	55.2
Mature stage	1.0850	6.40	195.8	54.5
Beta amylose	1.1690	15.10	214.0	60.6

BALDWIN (4), but the beta fraction of the soluble polysaccharides was not affected by this treatment. The results of further studies of the physical and chemical properties of these preparations are tabulated in tables III and IV. The viscosities, conductivities, and specific rotations of the beta

TABLE IV

COMPARISON OF CHEMICAL PROPERTIES OF BETA FRACTION OF SOLUBLE POLYSACCHARIDES AND BETA AMYLOSE FROM SWEET CORN STARCH

MATERIAL	P_2O_5	CONVERSION TO GLUCOSE ON HYDROLYSIS	MELTING POINT OF OSAZONES FROM HYDROLYZED SAMPLES	TOTAL NITROGEN
	%	%	° C.	%
Beta fraction of soluble polysaccharides				
Milk stage	0.0013	97.96	205.9	0.01*
Dough stage	0.0000	98.37	204.6	0.07*
Mature stage	0.0045	94.50	210.6	0.05*
Beta amylose	0.0042	92.88	209.4	0.006

* Single determination.

fraction of the soluble polysaccharide preparations are less than those of beta amylose. The phosphorus content of the beta fraction from the mature stage and beta amylose is the same, and from the specific rotation of hydrolyzed samples and the melting point of osazones it is evident that the beta fraction of the soluble polysaccharides is converted to glucose on hydrolysis, as is beta amylose.

COMPARISON OF PROPERTIES OF ALPHA FRACTION OF SOLUBLE POLYSACCHARIDES AND ALPHA AMYLOSE

The water soluble polysaccharides from the milk, dough, and mature stages contained approximately 22, 26, and 45 per cent. of the alpha fraction respectively, while the percentage of alpha amylose in the starch was approximately 14 per cent.

The properties of the alpha fraction and alpha amylose which have been compared are shown in tables V and VI. The iodine color of alpha amylose was violet while the iodine color of the alpha fraction was blue. As the corn matures the alpha fraction of the soluble polysaccharides loses in ash, phosphorus, and nitrogen; therefore the compound from mature corn had a higher percentage of conversion to glucose on hydrolysis. The optical rotation of the hydrolyzed samples varied with the stage of ripening of the

TABLE V

COMPARISON OF ASH, PHOSPHORUS, AND NITROGEN CONTENT OF ALPHA FRACTION OF SOLUBLE POLYSACCHARIDES FROM SWEET CORN AND ALPHA AMYLOSE FROM SWEET CORN STARCH

MATERIAL	ASH	P_2O_5	TOTAL NITROGEN
	%	%	%
Alpha fraction of soluble polysaccharides			
Milk stage	0.113	0.674	3.33
Dough stage	0.080	0.074	0.94
Mature stage	0.017	0.025	0.19
Alpha amylose	0.800	0.164	0.26

corn from which they were prepared. However, if the weights of hydrolyzed samples were corrected for their percentage of conversion to glucose, the optical rotations approximated that of d-glucose, with the exception of the material from the milk stage, which was lower. The alpha fraction of the soluble polysaccharides from the mature stage and alpha amylose contained a trace of extraneous fatty material, therefore it was either removed or corrected for, in determining the amount of fatty material freed by hydrolysis. The amount of this fatty material increased in the alpha fraction of the soluble polysaccharides as the corn matured. However, there was never as much present in these fractions as there was in alpha amylose. In making the determinations for the fatty material freed by hydrolysis, a flocculent precipitate occurred during the hydrolysis. This was collected

TABLE VI

COMPARISON OF PROPERTIES OF HYDROLYZED ALPHA FRACTION OF SOLUBLE POLYSACCHARIDES FROM SWEET CORN AND HYDROLYZED ALPHA AMYLOSE FROM SWEET CORN STARCH

MATERIAL	CONVERSION TO GLUCOSE ON HYDROLYSIS	SPECIFIC ROTATION OF HYDROLYZED SAMPLES AT 25° C.	MELTING POINT OF OSAZONES FROM HYDROLYZED SAMPLES	FATTY MATERIAL LIBERATED BY HYDROLYSIS
	%	%	° C.	%
Alpha fraction of soluble polysaccharides				
Milk stage	76.27	36.41	206.2	0.01
Dough stage	90.23	51.32	204.0	0.03
Mature stage	91.90	55.25	204.6	0.14
Alpha amylose	92.25	55.93	204.4	0.79

on a filter and washed free of acid before extracting with ether. The alpha fractions of the soluble polysaccharides from the milk and dough stages contained an abundant amount of this flocculent precipitate after hydrolysis, but when this was collected on a filter and washed it was found to be water soluble.

Properties of water soluble polysaccharides obtained from Evergreen sweet corn in milk stage

The water soluble polysaccharides from the Evergreen sweet corn were prepared in the same manner as described for the Hopeland sweet corn. The properties of the beta fractions of the soluble polysaccharides were very similar to those of the Hopeland fractions, with the exception that the Evergreen fractions had a higher conductivity.

The properties of the alpha fraction of the soluble polysaccharides from the Evergreen corn were similar to those of the Hopeland alpha fraction in the milk stage, with the exception that the Evergreen material had more ash and more fatty material freed by hydrolysis.

Discussion

The data on the beta fraction of the soluble polysaccharides indicate that it is possible to obtain from sweet corn a preparation which will have fairly consistent properties from year to year. The change in properties of this fraction as the corn matures may be due to an increase in the chain length of the compound. Therefore an attempt was made to determine the molecular weights of the different preparations by the cryoscopic method, but it was not possible by this method to obtain consistent results. In comparing the properties of the beta fraction of the soluble polysaccharides with those of beta amylose, it was evident that the physical properties of these two compounds are different. The former was readily soluble in cold water and gave an opalescent solution while the latter was difficultly soluble in cold water and gave a water-clear solution. The beta amylose had a greater viscosity, conductivity, and optical rotation than the beta fraction of the soluble polysaccharides. The chemical data indicate that these two compounds differ only in their physical properties. The specific rotation of the beta amylose was higher than that reported by other investigators (**4, 24**) working with beta amylose prepared from other kinds of starch.

The solubility of the alpha fraction of the soluble polysaccharides and alpha amylose was similar. Both compounds were readily soluble before electrodialysis; however, after repeated electrodialysis it was exceedingly difficult to get either compound into a stable suspension. Owing to this fact such physical measurements as viscosity, conductivity, and optical rotation would be of doubtful value.

The properties of the two fractions of the water soluble polysaccharides which have been investigated are not similar to any of the dextrins resulting from starch hydrolysis, as reported by SAMEC (**21**). Some dextrins resulting from direct hydrolysis of starch have been reported by BAKER (**3**) and by LINTNER and DÜLL (**15**) as having specific rotations which were very similar to that of the beta fraction of the soluble polysaccharides; however, the other properties reported by these investigators for their dextrin preparations were quite different from the properties of the alpha or beta fractions of the soluble polysaccharides. LINK (**14**) isolated and purified a dextrin from the radicle and plumule of the young corn plant. This dextrin was not similar to either fraction of the soluble polysaccharides found in sweet corn endosperm.

LAMPE and MEYERS (**13**) have studied the development of the endosperm of sweet corn microchemically, and report that globules first form and that carbohydrate grains which give characteristic starch reactions may or may not form within these globules. They advance the theory that these globules contain the water soluble polysaccharides. This idea was later given more strength by LAMPE (**12**). These investigators also report that they found no evidence of a reversal or a hydrolysis after the carbohydrate grains were once formed.

In the light of these recorded observations and the fact that the present investigation shows that the water soluble polysaccharides contain two fractions which in some respects are similar to sweet corn starch components, it is possible that the water soluble polysaccharides present in sweet corn are the units for the formation of starch grains.

Summary

1. The water soluble polysaccharides present in sweet corn endosperm were isolated and divided into two major fractions by electrodialysis. One fraction migrated to the positive electrode and was deposited as a gelatinous mass around the positive membrane while the other fraction always remained in suspension during electrodialysis. These fractions have been tentatively named alpha and beta, respectively.

2. The physical and chemical properties of these fractions of the soluble polysaccharides from corn in the milk, dough, and mature stages were compared with each other and with alpha and beta amylose prepared from sweet corn starch.

3. As the corn matured many of the properties of the alpha and beta fractions of the soluble polysaccharides approached those of alpha and beta amylose of starch but they departed widely from those that have been described for dextrins.

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SEASONAL MARCH OF CARBOHYDRATES IN *ELYMUS AMBIGUUS* AND *MUHLENBERGIA GRACILIS*, AND THEIR REACTION UNDER MODERATE GRAZING USE

EDWARD C. MCCARTY

(WITH THREE FIGURES)

Introduction

In a study of the western wheat grass, *Agropyron smithii*, the seasonal march of the total concentration of the sugar and starch fractions was found to vary inversely with the rate of growth of the herbage. The storage of these carbohydrates occurred during the declining phase of herbage growth, near the close of the annual growth cycle (**2**). A subsequent study of the seasonal march of the carbohydrates of the needle grass, *Stipa pulcra*, showed inverse proportionality between the rate of herbage growth and the total concentration of the sugar and starch fractions, in both the herbage and the stem bases and roots of the plant. Carbohydrate storage was also delayed until the closing phase of the annual growth cycle (**5**). In each of these grasses, one clipping treatment, applied at the onset of the interval of carbohydrate storage, resulted in a maximum diminution in these food materials in the stem bases and roots of the plant, at the close of the growing season.

In view of the economic importance of these facts in the management of range lands, it is obviously desirable to extend these studies to other grass species. The object of the present study therefore, was to determine: (1) the normal progress of the carbohydrate foods in the herbage and in the subterranean organs of the plant; (2) the period of food accumulation; and (3) the effect of a single harvest made at various stages of the annual growth upon the accumulation of carbohydrate food in the perennial organs of the plant.

The experimental plots were located at Ruxton, on the eastern slope of Pike's Peak, in Colorado. The immediate area was a grass covered hillside situated at the upper limit of the transition life zone, and with a gentle slope to the southeast. The soil was relatively deep on the slope, showing some rock outcrop and a loose admixture of gravel. The grasses studied were wild rye, *Elymus ambiguus* Vasey and Scribn., and a dropseed, *Muhlenbergia gracilis* Trin. These grasses were predominant on the area although there were other grasses and a sparse population of low herbs present.

At weekly intervals height growth data were secured by measuring 50 shoots, selected at random over the area. The dry weight increment was determined by drying the shoots in a ventilated oven at 65° C. (table I). Upon plotting these data, cumulative curves, sigmoid in character, were

TABLE I

HEIGHT, GROWTH, AND DRY WEIGHT OF *ELYMUS AMBIGUUS*; 1930

DATE	AVERAGE HEIGHT GROWTH	PERCENTAGE	AVERAGE DRY WEIGHT	PERCENTAGE
	cm.	%	*gm.*	%
June 24	30.8	52.5	0.130	11.8
July 3	36.0	61.4	0.218	19.8
July 10	36.5	62.4	0.221	20.1
July 18	38.7	63.0	0.225	20.4
July 30	45.7	78.0	0.319	29.0
Aug. 7	54.3	92.6	0.440	40.0
Aug. 15	57.1	97.6	0.649	59.0
Aug. 27	57.8	98.8	0.703	63.0
Sept. 4	58.6	100.0	.....	.....
Sept. 18	.....	.....	1.100	100.0

obtained. Rate curves were constructed by drawing lines tangent to the cumulative curves at convenient points, and computing the tangent of the angle made by the convergence of these lines upon the x-axis (1), (table II; figs. 1, 2).

The herbage samples as prepared for analysis consisted of the entire shoots, clipped 1 inch from the soil surface. The basal organs, consisting of stem bases, rhizomes, and roots, were combined for the purpose of analysis. Upon collection and preparation, these respective samples were placed in a ventilated oven and dried for a period of 24 hours at 65° C.

TABLE II

RATE OF GROWTH OF *ELYMUS AMBIGUUS*

HEIGHT GROWTH		DRY WEIGHT	
DATE	$\frac{Y}{X}$	DATE	$\frac{Y}{X}$
June 14	1.10	June 14	0.30
June 24	1.50	June 24	0.52
July 4	0.32	July 4	0.27
July 14	0.08	July 14	0.04
July 24	0.10	July 24	0.62
Aug. 3	2.04	Aug. 3	1.30
Aug. 13	0.52	Aug. 13	1.20
Aug. 23	0.10	Aug. 23	0.28
Sept. 2	0.00	Sept. 2	2.40
		Sept. 12	0.60
		Sept. 18	0.00

After grinding, the samples were dried for an additional 24 hours at the same temperature. The sugars were then extracted with alcohol. The residue remaining after the extraction was ground to a fine powder in a ball mill, and the starch fraction obtained by salivary digestion. The suspension was filtered through quantitative paper in a Büchner funnel. From this residue the hemicellulose was obtained by hydrolysis, using 2.5 per cent. concentrated hydrochloric acid. All solutions were cleared with basic lead acetate. The reductions were carried out as described in a previous paper (3).

Observations

Growth of the plants

The annual growth of a perennial range grass shows two general periods, characterized respectively by the development of the leafy shoots and by the lengthening of the flower stalks. The growth of the vegetative shoots is at first accelerated until the maximum rate is reached, after which the rate of growth declines. During this interval the inflorescence is differentiated from the apical meristem, thus initiating the second period of growth. The growth of the flower stalks is likewise accelerated, and as the rate declines, the fruit ripens and the plant reaches maturity.

In the wild rye grass the growth of the flower stalks progressed very slowly at first, and the heads appeared generally over the plots June 20. Following the change in acceleration of the rate of growth of the flower stalks, the dry weight of the plant became greatly accelerated (fig. 1). Depression in the rate of increase in dry weight was apparently correlated with flowering and the production of fruit, a condition found also in *Avena fatua* (3). As in this latter grass, the dry weight lagged behind the increase in height growth. Although the height growth had ceased on September 4, further increase in dry weight was indicated in the collection of September 18.

The growth of adventitious roots was observed July 30. This growth was completed in perhaps two weeks, after which the growth of secondary shoots began. The occurrence of new root and shoot growth during the declining phase of the annual growth cycle shows agreement with *Stipa pulcra* (5). Alternation in growth was found also in the study of *Avena fatua* (3). Parker and Sampson report root growth to be more active during the "autostatic" phase in *Stipa pulcra* and *Bromus hordeaceus* (4).

The annual growth of the plant was presumed to have been completed by September 18. At this time the fruit was in process of dissemination and the plants had assumed a characteristically mature aspect. There had also been a hard frost prior to this date.

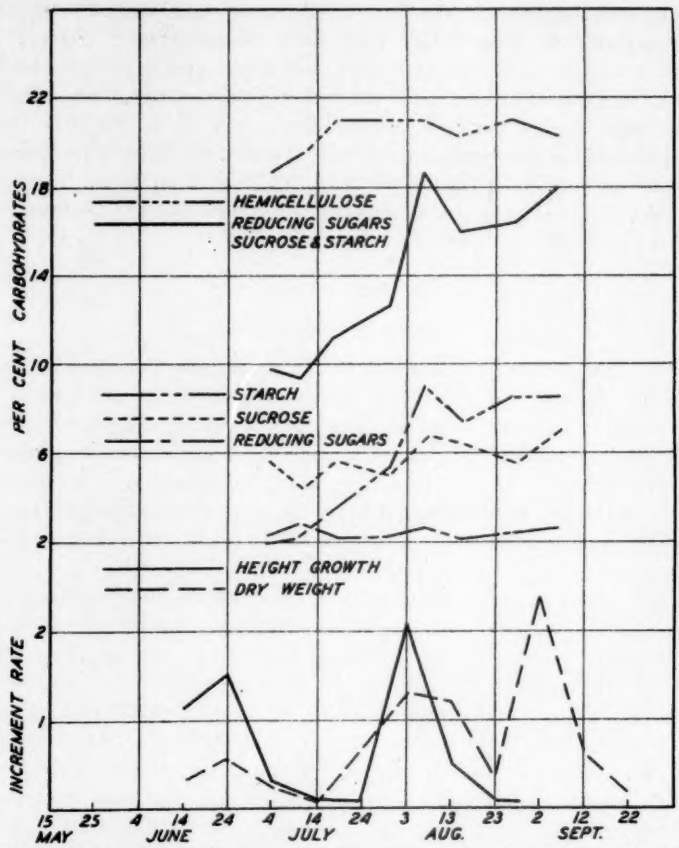

FIG. 1. *Elymus ambiguus*: upper, behavior of sugar, starch, and hemicellulose fractions in herbage of untreated plants (table IV); lower, rate of growth of the leafy shoots in height and their increase in dry weight (table II).

The principal growth features of *Muhlenbergia gracilis* were similar to those of *Elymus ambiguus*, although there were some differences in the time of appearance of the flower stalks and in the maturity of the fruit. Root growth, however, practically coincided in both grasses.

GROWTH OF THE PLANTS UNDER USE

Moderate use was approximated by clipping, one harvest being made of each plot successively as shown in table III. In all groups clipped during the period of vegetative activity some renewed growth occurred. The

renewed growth following clipping was not disturbed by any further treatment, and on September 6 the stem bases and roots were collected and

TABLE III

AVERAGE HEIGHT GROWTH OF CLIPPED PLOTS OF *ELYMUS AMBIGUUS*, 1930

PLOT	DATE OF HARVEST	AVERAGE HEIGHT BEFORE CLIPPING	AVERAGE HEIGHT SEPT. 6	TOTAL AVERAGE HEIGHT GROWTH
		cm.	*cm.*	*cm.*
1	June 24	30.8	31.6	62.4
2	July 3	36.0	24.8	60.8
3	July 10	36.5	19.8	56.3
4	July 18	38.7	17.2	55.9
5	July 30	45.7	8.0	53.7
6	Aug. 7	54.3	Appreciable	
7	Aug. 27	57.8	Appreciable	

prepared for analysis. Growth was renewed immediately following the harvest of plot 1, on June 24, but was somewhat delayed in the plot harvested on August 7. Whereas renewed growth was vigorous and immediate in plot 1, there was a progressive decline in the amount of growth made following the clipping of each successive plot.

If the total average height growth for the season is considered to be the sum of the average height growth at the time of clipping and the average height growth at the final harvest of each plot, there is an apparent increase in this factor when comparison is made with the untreated plants (tables III, I). A single clipping may both delay and diminish tillering, however; also, if flower stalks are produced, their diameters and ears of grain may be decidedly smaller than in the untreated plants (3). The yield following clipping is therefore lower than in the untreated plants.

MARCH OF THE CARBOHYDRATES

HERBAGE.—The concentration trends of the carbohydrates in the plant may be shown advantageously by means of the curve representing the sum of the reducing sugars, sucrose, and starch fractions (figs. 1, 2). In general the trend is from low concentrations in the early part of the growing season to high concentrations, the maximum occurring during the declining phase of growth at the close of the annual growth cycle. The increase in these carbohydrates between June 24 and the close of the season was more than 8 per cent., based upon the dry weight of the plant. The greatest change in concentration was found in the starch fraction, which increased rapidly following the turning point in growth rate of the vegetative shoots. The fluctuations in the sucrose level were smaller than those of the starch

fraction, but in both the trend was from low concentrations early in the growth cycle to high concentration at the close of the growing season. Only very slight changes occurred in the level of the reducing sugars throughout the period of observation. The variations in the acid hydrolyzable fraction, hemicellulose, were less than those of the starch fraction. A decline in the hemicellulose concentration, however, is shown at the time of flowering and the development of the fruit (table IV, fig. 1).

TABLE IV

REDUCING SUGARS, SUCROSE, STARCH, AND HEMICELLULOSE IN HERBAGE OF *ELYMUS AMBIGUUS*, EXPRESSED AS PERCENTAGE OF REDUCING SUGAR, 1930

SAMPLE NO.	DATE OF HARVEST	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	STARCH	REDUCING SUGARS, SUCROSE, AND STARCH	HEMICELLULOSE
		%	%	%	%	%	%
447	July 3	2.30	5.57	7.87	1.88	9.75	18.67
449	July 10	2.77	4.49	7.26	2.14	9.40	19.47
451	July 18	2.30	5.57	7.87	3.48	11.35	21.07
453	July 30	2.30	4.96	7.26	5.32	12.58	21.07
455	Aug. 7	2.69	6.81	9.50	9.08	18.58	21.07
457	Aug. 15	2.14	6.40	8.54	7.40	15.94	20.27
459	Aug. 27	2.38	5.49	7.87	8.48	16.35	21.07
463	Sept. 6	2.61	6.89	9.50	8.48	17.98	20.27

TABLE V

REDUCING SUGARS, SUCROSE, STARCH, AND HEMICELLULOSE IN STEM BASES, RHIZOMES, AND ROOTS OF *ELYMUS AMBIGUUS*, EXPRESSED AS PERCENTAGE OF REDUCING SUGAR. CALCULATIONS MADE UPON AN ASH FREE BASIS; 1930

SAMPLE NO.	DATE OF HARVEST	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	STARCH	REDUCING SUGAR, SUCROSE, AND STARCH	HEMICELLULOSE	ASH
		%	%	%	%	%	%	%
446	June 24	2.06	3.35	5.41	2.68	8.09	25.68	4.68
448	July 3	1.61	3.93	5.54	4.95	10.49	25.68	4.69
450	July 10	1.64	3.77	5.41	5.87	11.28	27.38	4.72
452	July 18	0.99	3.12	4.11	5.77	9.88	23.60	4.43
456	Aug. 7	1.08	2.36	3.44	6.52	9.96	22.76	4.22
458	Aug. 15	1.20	2.98	4.18	5.98	10.16	22.76	4.29
460	Aug. 27	1.13	2.45	3.58	6.92	10.50	23.76	4.52
464	Sept. 6	1.17	3.29	4.46	8.26	12.72	22.80	4.55
482	Sept. 18	1.17	4.63	5.80	8.26	14.06	22.80	4.59

STEM BASES, RHIZOMES, AND ROOTS.—The reducing sugars, sucrose, and starch in the stem bases, rhizomes, and roots showed trends similar to those in the herbage (table V, fig. 2). Low concentrations prevailed during the earlier phases of herbage growth, and maximum levels occurred with the decline in the growth rate at the close of the growing season. As in the herbage, the variations in the starch were greater than those obtaining in

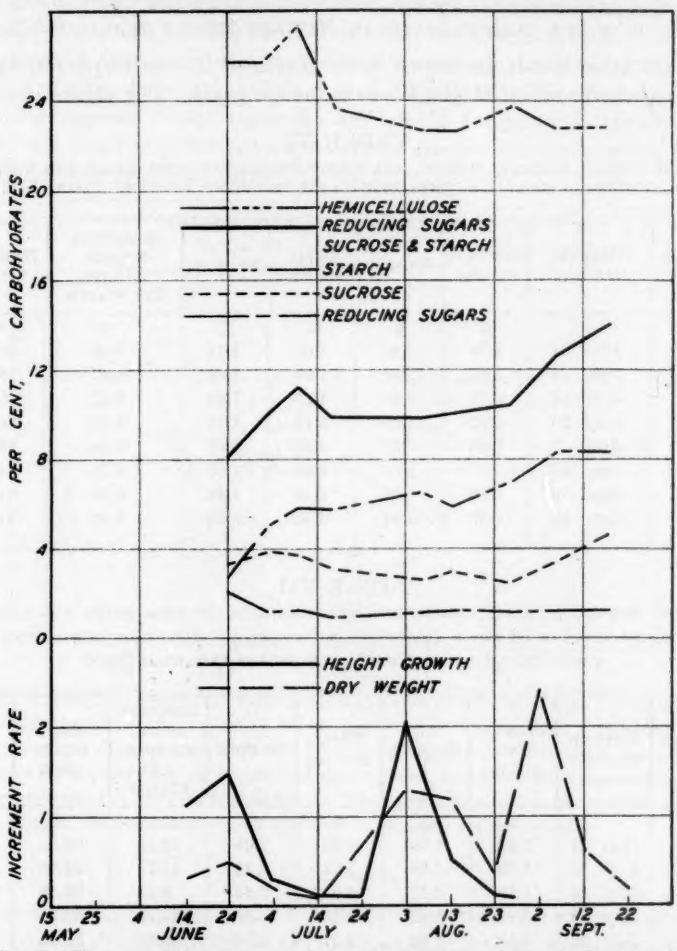

FIG. 2. *Elymus ambiguus*: upper, behavior of sugar and starch fractions in stem bases, rhizomes, and roots of untreated plants (table V); lower, rate of growth of the leafy shoots in height, and their increase in dry weight (table II).

the sucrose, and in both fractions the concentration trends were from low values on June 24 to high values at the end of the annual growth cycle. The reducing sugars were maintained at a more or less constant level throughout the period of observation. The concentration of the acid hydrolyzable hemicellulose declined during the reproductive period, showing agreement with the behavior of this fraction in the herbage.

MARCH OF THE CARBOHYDRATES IN *MUHLENBERGIA GRACILIS*.—Essentially the same trends are shown in the march of the carbohydrates in the stem bases and roots of *M. gracilis* as in the rye grass. The sugars exceeded

TABLE VI

REDUCING SUGARS, SUCROSE, STARCH, AND HEMICELLULOSE IN STEM BASES AND ROOTS OF *MUHLENBERGIA GRACILIS*, EXPRESSED AS PERCENTAGE OF REDUCING SUGAR; 1930

SAMPLE NO.	DATE OF HARVEST	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	STARCH	REDUCING SUGARS, SUCROSE, AND STARCH	HEMICELLULOSE
		%	%	%	%	%	%
421	June 24	0.78	2.83	3.61	1.78	5.39	36.32
425	July 10	1.15	3.84	4.99	1.88	6.87	36.32
427	July 18	0.77	3.48	4.25	1.88	6.13	36.32
429	July 30	0.83	2.59	3.42	3.12	6.54	36.32
431	Aug. 7	0.86	2.56	3.42	2.66	6.08	33.92
433	Aug. 15	0.77	2.33	3.10	2.00	5.10	34.32
439	Sept. 9	0.86	4.13	4.99	1.36	6.35	34.32
441	Oct. 18	0.89	4.64	5.53	3.36	8.89	33.92

TABLE VII

REDUCING SUGARS, SUCROSE, STARCH, AND HEMICELLULOSE IN STEM BASES AND ROOTS OF TREATED PLOTS OF *ELYMUS AMBIGUUS*, EXPRESSED AS PERCENTAGE OF REDUCING SUGAR. CALCULATIONS MADE UPON AN ASH FREE BASIS; 1930

SAMPLE NO.	DATE OF HARVEST	REDUCING SUGARS	SUCROSE	TOTAL SUGARS	STARCH	REDUCING SUGAR, SUCROSE, AND STARCH	HEMICELLULOSE	ASH
		%	%	%	%	%	%	%
466	June 24	1.97	3.69	5.66	7.08	12.74	26.33	6.79
468	July 3	1.39	3.88	5.27	5.44	10.71	24.96	9.47
470	July 10	1.19	2.72	3.91	5.32	9.23	25.46	7.46
472	July 18	0.99	2.81	3.80	6.30	10.10	25.41	7.40
474	July 30	0.95	2.62	3.57	6.76	10.33	25.65	7.78
476	Aug. 7	0.92	2.00	2.92	7.18	10.10	25.74	8.03
480	Aug. 27	1.04	2.39	3.43	5.70	9.13	23.47	6.64

the starch fraction in amount. The greater part of the sugar fraction was found to be sucrose, however, and this sugar is probably the more important storage product. The accumulated foods are laid down during the closing phase of the annual growth cycle, showing agreement with the wild rye grass (table VI).

CARBOHYDRATE CONCENTRATION IN CLIPPED PLOTS.—As previously stated, only one clipping was made of each plot, and the stem bases and roots were removed September 6. The relative values of the several carbohydrate fractions were in agreement with those in the untreated plants. As in the untreated plants, the starch values were higher than either sucrose or the reducing sugars. Based upon the amount of accumulated carbohydrate foods, the series is progressive, the largest amount being contained in the group harvested June 24 (table VII, fig. 3).

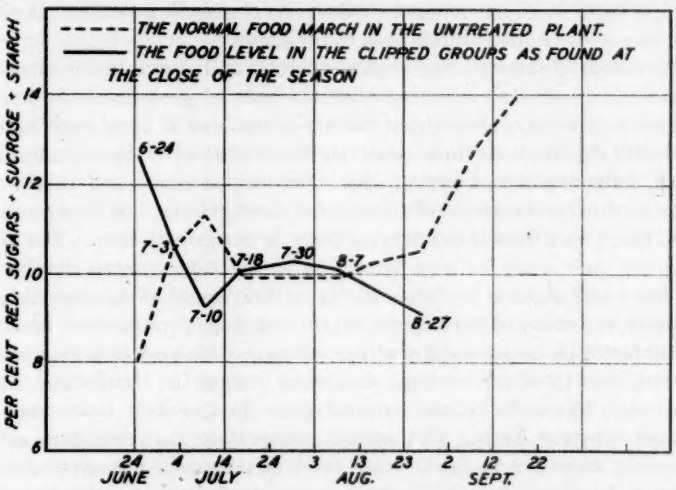

FIG. 3. *Elymus ambiguus*: total accumulation of sugar and starch fractions in the clipped groups, as found at close of annual growth cycle (table VII). The dates show when the single clipping treatment was applied in each group. Following these respective dates no further clipping treatments were applied, and the stem bases and roots of each group were collected on September 6. The curve representing the normal behavior of the total of the sugar and starch fractions is repeated for convenience.

Discussion

The annual growth cycle of *Elymus* is characterized by series of developmental steps, each occurring in sequence, each involving meristem activity, and each drawing upon the available carbohydrate foods of the plant. In the order of their occurrence, these steps comprise the development of the leafy

shoots, the initiation of the flowers, the elongation of the flower stalks, the growth of new adventitious roots, and the development and maturity of the fruit.

Although the photosynthetic area of the plant increases rapidly during the early period of herbage growth, utilization of the carbohydrate foods in respiration and in tissue building is apparently very great in proportion to the food making capacity of the herbage. Moreover, tillering occurs during this early period and the rate of growth is progressively accelerated. Carbohydrate utilization therefore continues at equal pace with the increase in leaf area and the synthesis of carbohydrate foods, and in consequence low concentrations prevail in the herbage during the early period of vegetative growth. The increase in the carbohydrate concentration coincident with the change in growth acceleration appears to be related to a decline in the rate at which these foods are utilized in the several growth processes, as well as to the increased photosynthetic area of the plant.

The march of the reducing sugars, sucrose, and starch in the stem bases and roots is inversely correlated with the rate of growth of the herbage. Previous studies have shown that the accumulations of these carbohydrates are greatly depleted within a relatively short time after the resumption of growth, following a rest period (5). Low values continued in the basal structures during the period of accelerated shoot growth, but increases in the several fractions followed the turning point in the growth rate. The inverse correlation prevailing between growth and the carbohydrate level in the stem bases and roots is evidence of the utilization of these materials at a rate quite in excess of their production, and hence production becomes a limiting factor in the several growth processes. Root growth is seen to alternate with growth of the herbage, occurring during the "autostatic" phase of growth. The carbohydrate concentration in the stem bases and roots remained constant during root growth, suggesting that meristem activity in the roots, as well as in the herbage, tends to monopolize the available food supply of the plant (3). With the conclusion of these growth phenomena, food accumulation was effected, the upturn of the concentration beginning on August 27 (table V, fig. 2).

As shown by the average height of the plants (table III), the most complete recovery was made in the group clipped on June 24. The amount of renewed growth made by each succeeding group decreased progressively, being only appreciable in the group clipped on August 27. Since the increment values in the untreated plants were greatest during the months of June, July, and August (table I), the external environmental factors may be presumed to have been favorable during these months. The amount of growth made by the several groups was proportional to the number of days remaining in the growing season following the clipping of each group (table

III). Food accumulation in the treated plants, as in the controls, occurs during the declining phase of growth, and both treated and untreated plants tend to reach this "autostatic" growth phase at the same time (2). Food accumulation in *Elymus ambiguus* agrees with that in *Agropyron smithii*, therefore, and is correlated with the amount of herbage present at the onset of the period of accumulation (2).

Renewed growth following clipping or grazing must be made at the expense of the accumulated carbohydrate foods in the stem bases and roots of the plants. Such growth is invariably followed by a diminution in the seasonal level of these foods in the storage organs (5, 3). This decline in the seasonal carbohydrate level tends to be proportional to the rate of growth at the time the clipping treatment is applied (3). Furthermore such diminution represents a loss of potential energy and imparts some injury to the plants. The contrast between the accumulated foods in the treated and in the untreated plants is shown graphically in figure 3.

There is reason to know that such diminution in carbohydrate foods as is found in the several groups of treated plants does not necessarily result in death of the plant. The amount of these foods found in the group clipped on August 27 is sufficient to promote fair growth during a subsequent season. Such diminutions serve to prevent maximum yields, however, and to lessen the number of fruit stalks produced during the subsequent season's growth. Again the decline in food level resulting from use tends to be cumulative when treatment is repeated from year to year.

Although the longer period of growth between the clipping of the first group on June 24 and the final upturn of the food level in the stem bases and roots yielded the most favorable results in the process of accumulation of carbohydrate foods in the stem bases and roots of the plants, this fact cannot be construed to favor early use of range lands. Further carbohydrate studies have shown specific injuries imparted by clipping or grazing during the first few weeks of seasonal growth. On the other hand the total annual growth of herbage is not essential to the production and accumulation of maximum amounts of carbohydrate foods. Moreover the results of the treatments applied to the several groups show the value of the late seasonal growth in the process of carbohydrate accumulation in the stem bases and roots of the plants.

Summary

1. The grasses *Elymus ambiguus* and *Muhlenbergia gracilis* were studied in their native habitat.

2. The seasonal march of the carbohydrates, as shown by the sum total of the sugars and starch, was found to be in inverse ratio to the rate of growth of the herbage.

3. Secondary growth of roots and shoots occurred during the closing phase of the growth cycle, and the carbohydrate concentration in the stem bases and roots remained practically constant during this growth activity.

4. The accumulation of carbohydrate foods was completed some time between September 7 and 18. Much of this food was apparently elaborated by the secondary herbage growth.

5. In the treated plants the highest and the lowest concentrations of the accumulated carbohydrate foods were found respectively in the group clipped June 24 and August 27, the latter being approximately at seed maturity.

6. In the clipped groups, the amount of herbage growth made subsequent to treatment and the concentration of the accumulated carbohydrates were roughly proportional to the number of days between the date of the clipping treatment and the end of the annual growth cycle.

The field work incident to the preparation of this paper was made possible by Dr. FREDERIC E. CLEMENTS, who also placed the facilities of the Alpine Laboratory at the disposal of the writer. The final collections of samples were made by Mr. LEONARD JOHNSON. Mrs. E. C. MCCARTY also assisted, both in the field and in the laboratory.

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GROWTH AND SEASONAL CHANGES IN COMPOSITION OF OAK LEAVES

ARTHUR W. SAMPSON AND RUDOLF SAMISCH

(WITH FOUR FIGURES)

Introduction

Study of growth and of seasonal changes in composition of leaves of certain western species of oak was undertaken as part of an investigation to determine forage values and certain management phases of the oak associations. An attempt was made to record seasonal changes in the major constituents of the leaves. The results are expressed (1) on a relative basis, namely, that of dry weight and of unit leaf area, respectively, and (2) on an absolute basis of leaf unit. The paper discusses the merits of the two bases for a study of this character.

Methods

Leaf samples were collected from *Quercus gambelii* Nutt. and *Q. kelloggii* Newb., both deciduous. *Q. gambelii*, a scrub form, occupies foothills and low mountains from southwestern Wyoming through Mexico and Arizona (**8**), whereas *Q. kelloggii* (**6**) is essentially a California species, occurring between elevations of 500 to 8000 feet throughout the length of the state (**3**).

Leaf samples of *Q. gambelii* were obtained from the same clumps during the entire growing seasons of 1930 and 1931, respectively.[1] In addition to this, a series of leaf samples was collected in 1931 from another oak clump located about 1 mile from the first clump. The analytical data pertaining to the samples gathered on the same date from these clumps agreed closely. The values for the growth rate of *Q. gambelii* consist of the averages for the corresponding samples from the two selected clumps (table I). Since no measurements of leaf area were made during the season of 1930, the data in the following tables pertain to samples gathered in 1931 only. These data are believed to be representative, however, since the analytical results obtained from leaf samples collected in 1930 show the same trend as those procured a year later.

Leaf samples of *Q. gambelii* were taken at approximately monthly intervals. The first samples were collected in June, when the leafage had unfolded sufficiently to afford browse for grazing animals. Flowering had been completed and acorns were beginning to form. By September the fruits attained full size, and many were being cast before reaching maturity.

[1] The samples of this species were collected through the courtesy of the Director of the Intermountain Forest and Range Experiment Station, near Ephraim, in central Utah.

At that time autumn color was still absent in the leaves. By the end of October all the leaves and the acorns had abscissed. The last sample, obtained on October 24, had to be collected from the ground.

Leaf samples of *Q. kelloggii* were collected during the growing season of 1931, near St. Helena in Napa County, California. The first sample was obtained in July, when the leaves had reached about half of their maximum season's growth. In September the leaves were still green. On December 1, when the last sample was collected, the leaves had turned partially brown, only a portion along the midrib still being green.

The average dry weight and the average area per leaf were determined as follows: leaves in lots of 300 were counted and weighed. Fifty leaves were blue-printed and their area measured with a planimeter. The two sets of leaf samples obtained from the two clumps of *Q. gambelii* checked closely. The figures as reported in table I for the leaves of this oak represent averages of 600 and 100 leaves, respectively.

Rate of growth

The rate of increase in area and the increase of the leaf in dry weight are shown in table I.

TABLE I

GROWTH RATE OF *QUERCUS GAMBELII* AND *Q. KELLOGGII*

	TIME OF HARVEST				
	JUNE 21	JULY 3	AUG. 6	SEPT. 8	OCT. 24
	Q. gambelii				
Average area per leaf in square inches	1.09	2.84	3.43	3.92	4.52
Average dry weight per leaf in mg.	69	166	207	235	205
	JUNE 11	JULY 19	SEPT. 13	DEC. 1	
	Q. kelloggii				
Average area per leaf in square inches	5.78	8.09	10.3	10.3	
Average dry weight per leaf in mg.	432	646	658	649	

After a rapid growth rate during the first month under observation, the rate of expansion of leaves of *Q. gambelii* decreased measurably. The increase of leaf area, expressed in percentage of leaf area of each of the preceding samples, was 160 per cent. for the period between June 11 and July 2, 21 per cent. between July 3 and August 6, and 14 and 15 per cent.,

TABLE II

CONSTITUENTS OF DECIDUOUS OAK LEAVES IN GRAMS PER 1000 LEAVES
SAMPLES COLLECTED 1931

	Quercus gambelii					*Quercus kelloggii*			
	June 11	July 3	Aug. 6	Sept. 8	Oct. 24	June 21	July 19	Sept. 13	Dec. 1
Dry weight	69.4	166	207.5	233	205	432	646	658	649
Water	151.8	313.3	204.5	235	207	379	.....	.....	549
Ash	3.7	7.7	9.5	10.5	9.9	19.0	34.6	35.2	41.4
Crude protein	16.9	30.5	30.8	31.5	9.9	51.8	73.5	58.3	44.2
Crude fiber	10.2	40.4	44.0	43.4	48.6	76.1	121.2	129.0	127.2
Ether extract	0.8	4.5	5.5	8.4	8.9	10.6	18.7	24.8	26.8
N-free extract, by difference	37.8	82.9	117.7	139.2	127.7	274.5	398.0	410.7	409.4
SiO_2	0.23	0.51	0.95	1.36	1.57	2.07	4.97	6.91	12.28
CaO	0.40	1.76	2.59	3.02	3.50	5.48	8.26	8.22	12.01
P_2O_5	0.85	1.32	1.35	1.74	1.31	2.76	5.23	4.80	4.86
K_2O	1.36	3.05	3.26	3.34	2.60	5.09	9.18	9.87	6.36
Na_2O	0.13	.....	.....	.....	0.31	0.48	.....	0.94	0.96
Cl	0.01	.....	.....	.....	0.05	0.07	.....	0.13	0.17

respectively, for September and October. In *Q. kelloggii* a corresponding increase of 40 per cent. was observed during the first month, compared with a total increase of 27 per cent. during the following two months. There was no further increase in area after September 13. Apparently the stage of growth in *Q. kelloggii* was somewhat more advanced at the time the experiment was started than was that of *Q. gambelii*.

A similar trend was also observed in the increase of dry weight per leaf. The decrease in weight of the last collected sample of *Q. gambelii* (October 24) may be attributed either to leaching or to translocation of nutrients from the leaves to the branches.

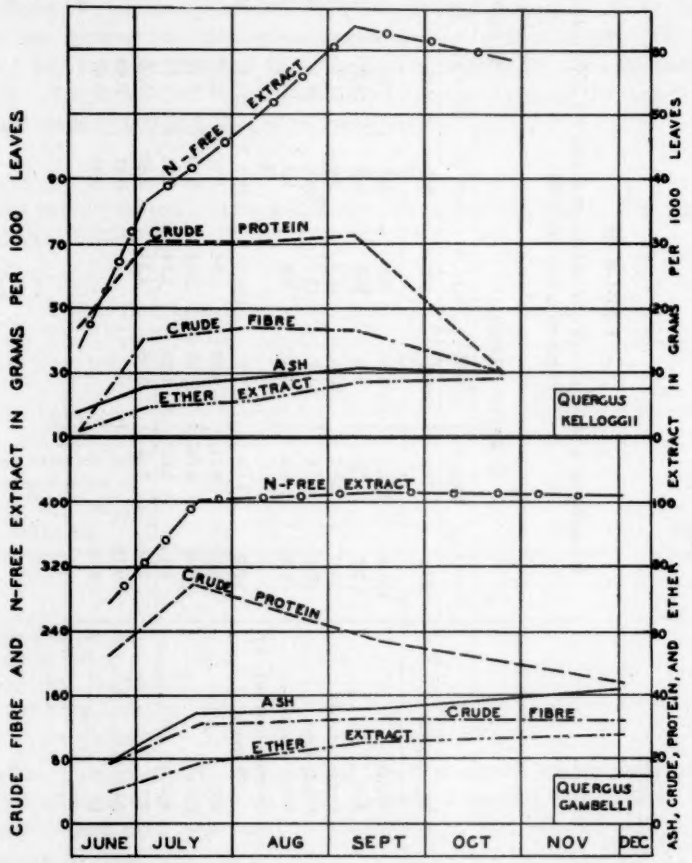

FIG. 1. Major constituents of oak leaves, expressed in grams per 1000 leaves.

On the basis of the growth data presented it was deemed advisable to distinguish three stages of leaf development: (1) the period of rapid growth, (2) the period of maturation, and (3) the period of senescence.

Chemical changes

The analytical methods used in this study will be published in detail elsewhere; with slight modification, they were those adopted by the Association of Official Agricultural Chemists.

In table II and figures 1 and 2, the analytical results are presented on the basis of grams per 1000 leaves. In table III and in figures 3 and 4, the

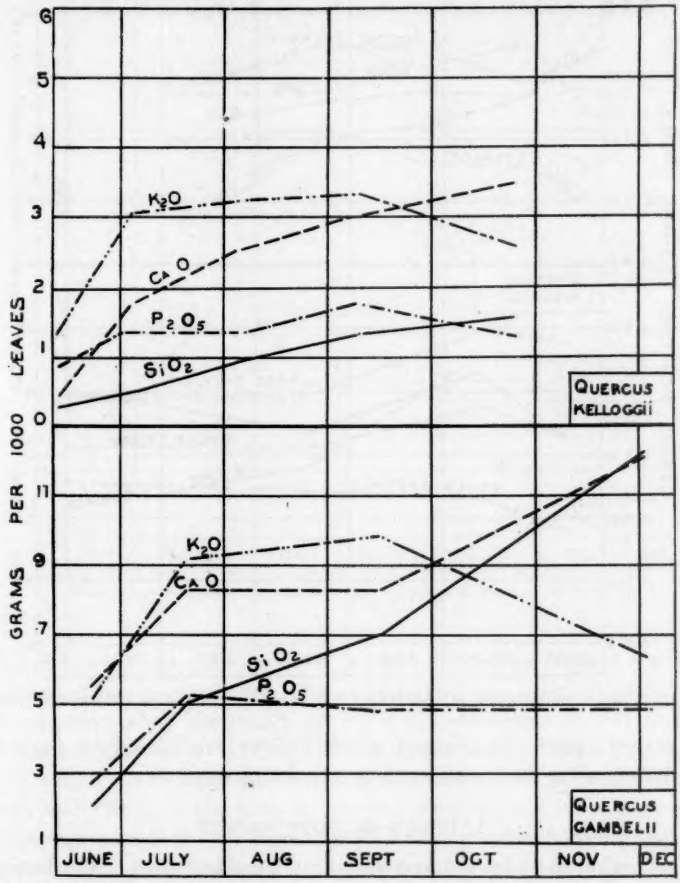

FIG. 2. Mineral constituents of oak leaves, expressed in grams per 1000 leaves.

data are expressed on a leaf area basis, namely, in grams per 100 square inches of leaf surface.

In the following discussion changes in constituents are shown for the three stages of leaf development. For convenience, the data presented for

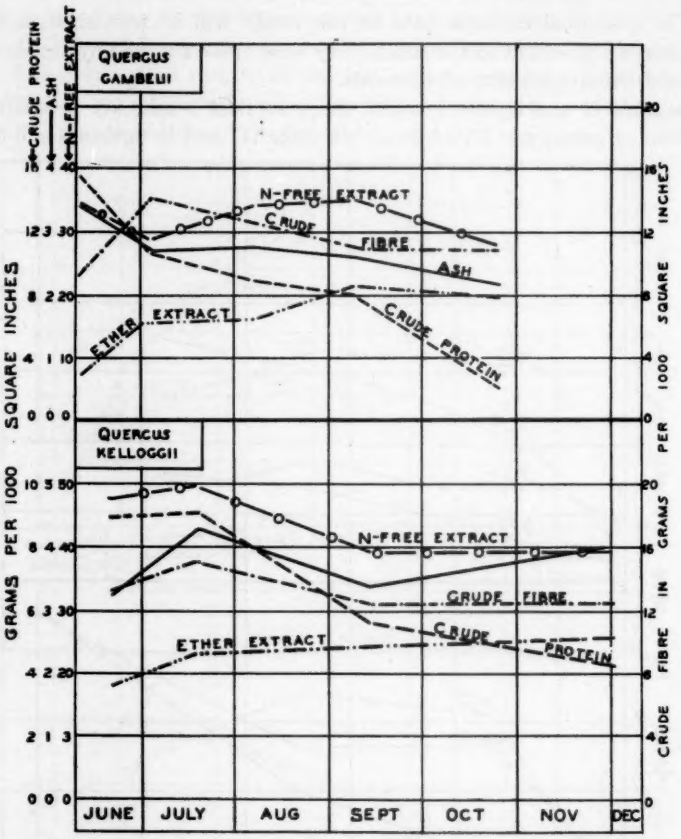

FIG. 3. Major constituents of oak leaves, expressed in grams per 1000 square inches.

Q. kelloggii under the heading which follows are placed in parenthesis immediately after the corresponding ones for *Q. gambelii*.

1. PERIOD OF RAPID GROWTH

This period includes the first month under observation. The basic data are given in table II and their relative trends are shown in figure 1.

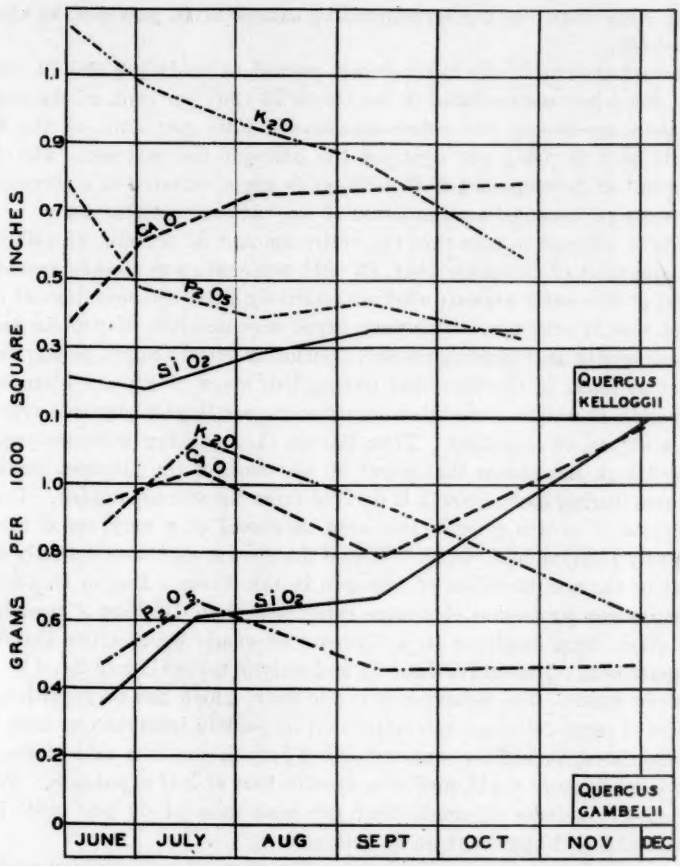

FIG. 4. Mineral constituents of oak leaves, expressed in grams per 1000 square inches of leaf surface.

The period of very active growth is characterized by a rapid accumulation of salts and carbohydrates. From table II it has been calculated that, within approximately one month after the first collection of leaf material, there was an increase per leaf of 108 (82.6) per cent. in ash; 80 (42) per cent. in protein; 298 (59) per cent. in fiber; 463 (76) per cent. in ether extract; and 119 (59) per cent. in nitrogen-free extract. Throughout the early growth period *Q. kelloggii* showed a slower rate of accumulation of the constituents just compared. This behavior may be explained by the fact that the first sample of *Q. kelloggii* was collected at a more advanced

growth stage than was the corresponding sample of *Q. gambelii*, as already pointed out.

Toward the end of the rapid growth period, as on July 3 and 19, respectively, there had accumulated in the leaves 73 (83) per cent. of the total of ash taken up during the entire season; 51 (70) per cent. of the ether extract; and 60 (97) per cent. of the nitrogen-free extract. The more advanced leaf development of *Q. kelloggii* is again reflected in a correspondingly more pronounced accumulation of the various constituents.

It is of interest to note that the entire amount of protein, 97 (100) per cent., and most of the crude fiber, 83 (94) per cent., was already present in the leaf at this early season; whereas relatively little accumulation of ether extract was in evidence. This very rapid accumulation of protein and of some ash constituents during the early period of growth might be attributed, in part at least, to the fact that during this stage of growth nitrogenous compounds, as well as some other constituents, are largely obtained from the storage organs of the plant. Thus BAUER (1), working with one-year-old oak seedlings, has shown that about 60 per cent. of the nitrogen intake of the leaves during early growth is derived from the storage organs. During this period of active growth, leaf area increased at a very rapid rate, as previously pointed out. In *Q. kelloggii* the growth rate was virtually equal to that of the accumulation of nitrogen in the leaves. But in *Q. gambelii* leaf expansion proceeded at a more rapid rate than did that of protein accumulation, thus resulting in a decrease of crude protein per leaf area. (Compare crude protein for June 11 and July 3, table III and fig. 3.)

CRUDE FIBER.—The behavior of crude fiber, which can be regarded as a measure of ligno-cellulose, resembles that of protein inasmuch as most of it is accumulated during the stage of active growth; but the rate of accumulation is much more rapid, and even exceeds that of leaf expansion. This is reflected by increase of crude fiber per unit area of 52 per cent. in *Q. gambelii* and of 13 per cent. in *Q. kelloggii*.

Morphologically, ligno-cellulose is a function of cell division and cell growth. An additional factor which has a bearing on the amount of ligno-cellulose in the leaves is the development and increase of the fibrovascular system and the lignification of the pedicel of the leaf.

NITROGEN-FREE EXTRACT.—This consists largely of sugars, hemicelluloses, and pectins. The expansion of photosynthetic surface was accompanied by the accumulation of these substances in the leaves. The increase of these constituents in *Q. gambelii* (Utah) did not quite keep pace with the rate of leaf expansion, whereas in *Q. kelloggii* (California) its accumulation exceeded the rate of growth.

ASH.—The ash constituents (table II, fig. 2), similar to the organic compounds, accumulated rapidly during the period of early growth. During

TABLE III

CONSTITUENTS OF DECIDUOUS OAK LEAVES IN GRAMS PER 1000 SQUARE INCHES
SAMPLES COLLECTED 1931

	QUERCUS GAMBELII					*QUERCUS KELLOGGII*			
	JUNE 11	JULY 3	AUG. 6	SEPT. 8	OCT. 24	JUNE 21	JULY 19	SEPT. 13	DEC. 1
Average area per leaf	1.09	2.84	3.43	3.92	4.52	5.78	8.09	10.35	10.27
Dry weight per 1000 square inches	64.3	58.4	60.4	59.4	45.4	74.7	80.0	63.6	63.2
Water	139.3	110.3	59.95	59.95	4.58	65.4	.....	.....	53.4
Ash	3.09	2.71	2.77	2.62	2.19	3.28	4.28	3.40	4.03
Crude protein	15.50	10.74	8.97	8.03	2.19	8.98	9.10	5.64	4.30
Crude fiber	9.36	14.21	2.81	11.08	10.87	13.18	14.91	12.48	12.40
Ether extract	0.73	1.58	1.60	2.14	1.97	1.84	2.32	2.39	2.61
N-free extract, by difference	34.62	29.16	34.25	35.53	28.27	47.42	49.39	39.69	39.86
SiO_2	0.21	0.18	0.28	0.35	0.35	0.36	0.61	0.67	1.19
CaO	0.37	0.62	0.75	0.77	0.78	0.95	1.04	0.79	1.17
P_2O_5	0.78	0.97	0.39	0.44	0.29	0.48	0.65	0.46	0.47
K_2O	1.25	1.07	0.95	0.85	0.58	0.88	1.13	0.95	0.62
Na_2O	0.12	.....	.....	.....	0.07	0.083	.....	0.091	0.093
Cl	0.009	.....	.....	.....	0.012	0.0012	.....	0.0012	0.0016

this period calcium increased 340 (51) per cent., potassium 124 (80) per cent., silica 122 (140) per cent., and phosphorus 55 (89) per cent. These great differences in rate of accumulation of the various ash constituents are seen more clearly when plotted on the basis of unit area (fig. 4).

While changes per leaf represent absolute values of accumulation or loss, a comparison of changes on a unit area basis involves two variables, namely, the rate of leaf expansion and the rate of intake of constituents. In *Q. gambelii* (fig. 2) there was a rapid accumulation of all elements in the individual leaf when compared on a leaf basis, whereas figure 4 shows that phosphorus, potassium, and even silica decreased in *Q. gambelii* when plotted on a per unit area. In other words, the rate of intake of these constituents was smaller than the rate of leaf expansion. On the other hand, where there was a slower growth rate, such as in *Q. kelloggii*, all elements were on the increase during this early period of growth, regardless of whether the data were calculated on the leaf area bases or on the basis of a single leaf.

Similar trends were obtained when the results were reported as percentage of dry weight, for rate of leaf expansion and rate of increase in weight happened to run parallel at this early stage of development. Data are frequently reported in the literature as percentage of weight, seldom on the basis of unit area. It is often difficult to gain a picture of the absolute accumulation of or loss of materials unless a given organ or plant is taken as a basis of reference. The importance of this point in interpretation of the analytical results has been strongly emphasized by WEHMER (9).

2. PERIOD OF MATURATION

This period covered the months of July and August. It was characterized by a substantially reduced rate of growth and by a slow increase in all constituents. It will be noted (fig. 1) that in *Q. kelloggii* resorption of nitrogen, and possibly of phosphorus (fig. 3), had already taken place.

In the leaves of *Q. gambelii* the smallest increase was in protein. Prior to this period most cells had ceased to divide. Enlargement of the leaves was proceeding at a somewhat more rapid rate than was the increase in those constituents. They showed, therefore, an apparent decrease when calculated on a unit area basis. The decrease of crude fiber per unit area was due to the relative increase of the area of the leaf proper, as compared with the veins and stem of the leaf, which are known to be comparatively rich in ligno-cellulose.

During this period (samples collected August 6, September 8 and 13) ether-soluble substances increased at a fairly rapid rate whereas crude fiber and potassium remained practically stationary in both species. Accumulation of nitrogen-free extract almost ceased in *Q. kelloggii* whereas it continued at a moderate rate in the leaves of *Q. gambelii*. Silica increased at

a constant rate in both species. This was also the case with calcium in the leaves of *Q. gambelii*. It seems peculiar, however, that in *Q. kelloggii* there was no intake of calcium for a period of two months.

Although silica is frequently regarded as an external contamination, this seems to be the case only to a limited extent with the material used in this investigation, which was carefully wiped before grinding. If the presence of silica were due merely to dust deposit, then it would hardly have showed fluctuation in the rate of deposition per unit area, as exhibited by the present material.

During the period of maturation certain constituents showed an apparent loss in the leaf, when compared on a leaf area basis with samples collected earlier in the season, despite the fact that in absolute amounts, *i.e.*, per leaf unit, these constituents had actually accumulated in the leaves or had at least remained stationary. Such is the case for crude protein in *Q. gambelii*, for nitrogen-free extract in *Q. kelloggii*, and for crude fiber in both species.

3. Period of senescence

This phase is characterized by a resorption of a number of constituents from the leaves of deciduous trees into branches, trunk, and roots. The early literature on the subject was discussed by Wehmer (**9**) and by Swart (**7**). More recently the translocation of nitrogen compounds from the leaves to other organs of trees has been reported by Combes (**2**) and by Lincoln (**4**).

In the present work, one leaf sample from *Q. kelloggii* and from *Q. gambelii*, respectively, was obtained during the period of senescence (tables II, III). The sample of *Q. gambelii*, collected October 24, consisting of dead leaves collected from the ground, showed a loss in protein, phosphorus, and potassium. This behavior might have been the result of leaching rather than of translocation of these constituents from leaves to twigs and branches.

On the other hand, the leaves of the sample of *Q. kelloggii*, collected December 1, also showed a loss in nitrogen, phosphorus, and potassium. These leaf samples were alive and had not abscissed. It is highly probable, therefore, that the losses in constituents previously enumerated were due to their resorption from the leaves and presumably not to leaching. In *Q. kelloggii* the amount of phosphorus in the leaves did not change between September 13 and December 1; but a significant portion of the phosphorus and the protein was translocated from the leaves between July 19 and September 13. Resorption of phosphorus and of nitrogen evidently started in this species at an earlier period than did that of potassium. On the other hand, silica and calcium continued to accumulate in the leaf whereas crude

fiber and ether extract accumulated slightly or remained at a stationary level.

Summary

1. Leaves of *Quercus gambelii* (Utah) and of *Q. kelloggii* (California) were collected at more or less regular intervals during the growing season. They were analyzed after their area and their dry weight had been measured.

2. Protein was found to have accumulated in the leaf at a very early stage of growth. Although no more nitrogen was taken up during the remainder of the season, leaf expansion continued, resulting in a reduction in protein content per unit area. In the latter part of the growth period there was an actual resorption of protein from the leaf into the storage organs.

3. Most of the crude fiber, like that of protein, was accumulated early in the growth period, after which it increased only very slowly. It decreased per unit area during the latter part of the season because of the more rapid leaf expansion during this period.

4. Ether-soluble substances accumulated continuously throughout the growing season.

5. The nitrogen-free extract increased very rapidly in the California species studied, and remained almost constant after the initial rise (July); whereas it continued to accumulate gradually throughout the season in the Utah species, at least as late as September.

6. Calcium and silica increased at a rapid rate throughout the growing period.

7. Potassium showed a rapid rise in spring, followed by a somewhat declining increase during the summer. This slow rate of increase during the summer, as compared with leaf expansion, resulted in a drop of potassium per unit area. There was a pronounced resorption of potassium in the fall.

8. Phosphorus showed an early peak in the California leaf samples, whereas this peak did not occur before September in the Utah species.

9. This study emphasizes that in order to follow seasonal changes in the *absolute* amounts of chemical constituents occurring in specific plant organs, or in entire plants, the data should correspondingly be expressed on the basis of a specific organ, such as the leaf, or the plant as a whole.

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MODIFICATION OF LEAF STRUCTURE BY X-RAYS

YAKICHI NOGUCHI

(WITH SIX FIGURES)

Introduction

The effect of x-rays upon seeds and seedlings very often causes abnormalities of form and changes in the internal structure of the plant. The cells of a treated radicle show elongation, great vacuolation or entire lack of protoplasm, and absence of nuclei from many cells (**1, 6**). Strong irradiation sometimes causes tumor-like tissues at the root tip, which give a bulbous and swollen appearance and which contain multinucleated giant cells (**4, 7**). Fasciations of stems, leaves, and flowers in the sunflower and bushy forms of young tomato plants occur after treatment (**1, 3**). Abnormality of leaves is a very common injury by x-rays: the leaves become asymmetric, distorted, pocked; light green areas intermingle with ordinary green as if the leaves were suffering from a mosaic disease (**1, 3, 10**).

Very few studies have been made on the structure of these abnormal leaves. The writer found that one investigator has reported the interference with the normal development of palisade cells and spongy parenchyma which takes place in such leaves (**3**).

Material and methods

Since Russian sunflower, *Helianthus annuus*, is very sensitive to x-rays and abnormality of vegetative parts can easily be produced by irradiation, this plant was selected for these experiments. Seeds were placed for about 24 hours in a moist chamber on a layer of cellucotton saturated with distilled water, and kept at a temperature of 25° C. Some investigators (**2, 5, 8, 9**) found that the sensitivity of seeds to x-rays is influenced by the amount of water they contain at the time of treatment; therefore it is necessary to measure the water content of the material. The average water content of air-dried seeds of *Helianthus* is 3.75 per cent. After keeping them in moisture for 24 hours this increases to 38.83 per cent. and the seeds show incipient germination. At this stage, after removing the pericarps, as uniform seeds as possible were selected for treatment and controls. The irradiation used in these experiments was made with an x-ray machine in the following set-up: 100 K.V. 5 ma. current, no screen, 30 cm. focal distance. Immediately after treatment both irradiated seeds and controls were planted in pots and kept under environmental conditions as much alike as possible, in the greenhouses of the University of Chicago.

Results

General aspects of leaf abnormality

The most common deformities presented by leaves after treatment are asymmetry of blade and distortion caused by small pockets. Light or dark green (occasionally both) areas intermingle with ordinary green, and, very rarely, even colorless portions appear along the margin of the leaf (fig. 1, *a–c*). With strong irradiations the first two or three leaves show a peculiar aspect, as if they were suffering from a mosaic disease (fig. 1, *d*).

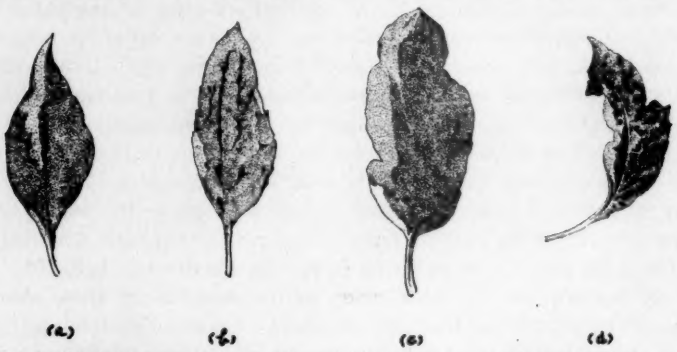

Fig. 1. Variations of color produced in leaves by x-ray irradiation: *a*, light green areas in ordinary green leaf; *b*, dark green scattered streaks; *c*, colorless area along the margin; *d*, mosaic diseased appearance.

An experiment has been performed with the time element as the only variant in the production of abnormalities in leaves. The seeds were divided into five series of ten seeds each, and irradiated for 5, 10, 15, and 20 minutes respectively, the remaining lot being unradiated, as control. Observations were made after ten weeks, when the plants were nearly mature. Table I records the abnormalities observed.

Histology of normal leaf

Cross sections of young leaves about 1.5 cm. long, from normal plants which were used as controls, were examined to determine the normal development of the tissues in the early stages. One or two layers of long but uniform palisade cells lie under the epidermis, and the space between the palisade tissue and the epidermis of the under surface of the leaf is filled with small cubic spongy parenchyma. All the cells in young tissue appear light green with compactly arranged chloroplasts. As the leaves grow the palisade cells become longer and the spongy parenchyma cells become angular in shape. The green elipsoidal chloroplasts are found

TABLE I

LEAF ABNORMALITIES APPEARING AFTER IRRADIATION OF X-RAYS: M, HEAVY MOSAIC DISEASED APPEARANCE; m, LIGHT MOSAIC DISEASED APPEARANCE; L, LIGHT GREEN AREAS INTERMINGLED WITH NORMAL GREEN; l, SMALL LIGHT GREEN AREAS INTERMINGLED; D, DARK GREEN AREAS INTERSPERSED WITH NORMAL GREEN; w, COLORLESS AREAS APPARENT; N, NORMAL

No. LEAVES	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
No. PLANTS	Series I (20 minutes' treatment)																	
1	M	M	the bud arrests growth															
2	M	M	Lw	L	l	l	ml	Ml	l									
3	M	M	L	D	l	l												
4	m	m	L	LD	l	lw	lw											
5	M	M	L	L	L	L	l	l										
6	M	M	L	L	L	L	L	L										
	All leaves asymmetric and distorted; four plants died from heavy injuries before the emergence of leaves																	
	Series II (15 minutes' treatment)																	
1	M	M	m	l	l	a side bud appears												
2	M	M	L	l	l	N	N	l	N									
3	m	m	L	L	the bud arrests growth													
4	M	L	L / N	L / N	L	two buds develop												
5	m	m	L	L	l	N	l	l	l									
6	M	M	m	m	L	L	l	l	l									
7	m	L	L	N	N	N	N	N	N	N	N	N	N	N				
	All leaves asymmetric; three plants died in seedling stage																	
	Series III (10 minutes' treatment)																	
1	L	L	L	l	l	l	N	N	N	N								
2	m	m	N	N	N	N	N	l	N									
3	L	L	l	l	N	N	N	N	N	N	N	N	N					
4	L	L	l	l	l	N	N	N	N	N	N	N	N					
5	m	m	L	l	l	N	N	N	N	N	N	N						
6	M	M	m	m	L	L	l	l	l	N	N	N	N	N				
7	m	m	D	d	N	N	N	N	N	N	N	N	N					
8	m	m	m	L	L	l	l	N	N	N								
9	M	M	L	L	l	l	l	N	N									
	All leaves slightly asymmetric and pocked; one plant injured by insects is excluded from the table																	
	Series IV (5 minutes' treatment)																	
1	m	m	l	l	N	N	N	N	N	N	N	N	N					
2	m	m	N	N	N	N	N	N	N	N	N	N						
3	m	m	l	N	N	N	N	N	N	N	N	N	N	N	N			
4	L	L	N	N	N	N	N	N	N	N	N	N	N					
5	m	m	m	m	N	N	N	N	N	N	N							
6	m	m	N	N	N	N	N	N	N	N	N	N	N					
7	m	m	L	L	L	l	l	N	N	N	N	N	N					
8	m	m	l	l	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	All leaves symmetric; two plants injured by insects																	

gathered closely along the inside cell wall. In the mature leaf (4 cm. in length) the arrangement of cells is very regular.

Histology of abnormal leaf

Colorless part.—The appearance of colorless parts is rare; it seems to be a severe injury since it was induced only in a series of strong irradiation. The structural aspect of the leaf is just like that of an ordinary variegated leaf. There is no disturbance of tissue in the green part, although the color is somewhat lighter, probably owing to the defective chlorophyll-absorbing power of the chloroplasts. On the boundary between colorless and green tissues, in most cases the abnormal development of both palisade and spongy cells is found in the colorless area. The palisade cells are, in some instances, unusually slender, frequently curved, smaller, and more spherical. Discrepancies often occur in place of the regularity of the normal palisade arrangement. No differentiation between palisade and parenchyma occurs in the center of the colorless section when x-ray dosage is severe. A few chloroplasts without chlorophyll are found in cells of colorless tissue.

Light green part.—Two kinds of structures are found in the light green section of the leaf. Sometimes the color is caused by the small quantity of chloroplasts in the cells; in such cases no change of structure occurs. Usually, however, the development of tissues, especially palisade, is abnormal, accompanied by a lack of chloroplasts. In the normal leaf palisade cells stand side by side very compactly, but in the abnormal leaf, owing to the irregular arrangement, small or large spaces appear between palisade cells (fig. 2). When the injury is considerably severe, the entire

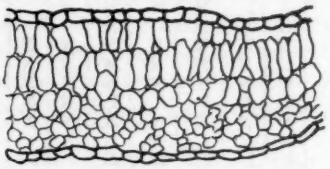

Fig. 2. Abnormal structure of light green parts. Small or large spaces appear between palisade cells owing to their irregular arrangement.

leaf appears light green with small irregular streaks of normal or dark green scattered over the surface, and the texture of the leaf becomes exceedingly coarse. Significant interference of development between palisade and spongy parenchyma is indicated in the light green areas of such leaves (fig. 3). The chloroplasts in the abnormal cells are more spherical and smaller than in the normal; they also seem to lack the normal power of absorption of chlorophyll.

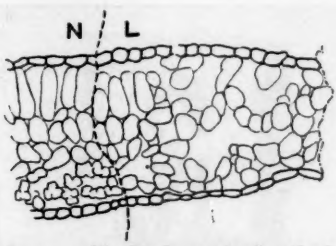

FIG. 3. Strong interference with development of palisade and parenchyma in the severely injured light green area: N, normal; L, light green parts.

DARK GREEN PART.—Dark green irregular streaks appear scattered over the leaf surface. This deep color is due to the extremely strong chlorophyll-absorptive power of the chloroplasts.

MOSAIC-DISEASED APPEARANCE.—The most interesting leaves were those showing the mosaic-diseased appearance. Distinct disturbance of development occurred in all tissues, and when the cross section of the leaf was observed under the microscope, unusual structures were frequently found. The arrangement of epidermal cells was more or less wavy or zigzag. Within a single microscopic field of vision the cells ranged from dark to normal to light green in color (fig. 4). Occasionally one or two layers

FIG. 4. Cross section of mosaic leaf showing dark, normal, and light green areas.

of colorless cells were found directly beneath the epidermis. From these observations it is readily concluded that the structure of the abnormal leaf is very complicated, and even in the least disfigured leaf several structural abnormalities will be found.

The general aspects of structure of the light and normal green areas are the same as those already mentioned (fig. 5, left, middle). The structure of the dark green areas, however, is entirely different from that of the dark green area described above.

The distinction between palisade and parenchyma tissues in most cases no longer exists. The deformed chloroplasts, clustered in groups of approximately six to eight, fill the cells and are a more bluish green. Frequently, moreover, the chlorophyll diffuses through the cell sap; thus the leaf appears even darker. The walls of the parenchyma cells are thin and less distinct. In the lower layer of the parenchyma coagulation of the

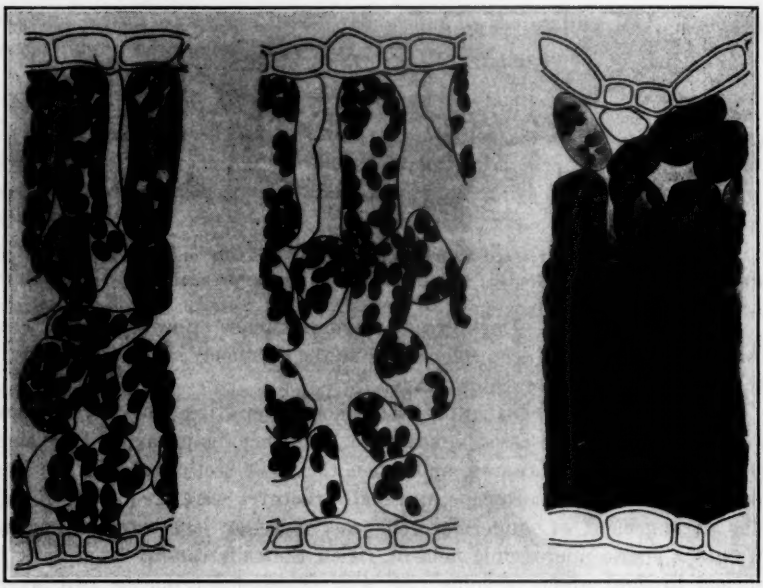

FIG. 5. Internal structures of mosaic leaf: left, normal; middle, light green; right, dark green portions.

chloroplasts occurs and the cell walls are even less distinct (fig. 5, right). Pockets are caused by the gradual or sudden interruption of a layer of palisade cells.

After the longest periods of irradiation a very peculiar structure is found in the leaf with the mosaic appearance. All of the cells become

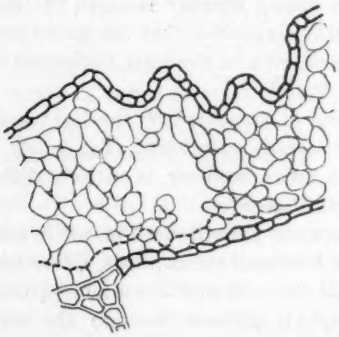

FIG. 6. Strong abnormality of structure after severe irradiation by x-rays. All the cells become globose and similar and are accompanied by one layer of colorless cells (under the broken line).

globular and similar and contain only a very small number of chloroplasts or none at all (fig. 6). The abnormality of structure can be seen even in the early stages of a treated leaf.

Effect of minimum dose of x-rays

It has been reported by several investigators that comparatively strong x-rays induce leaf abnormality, but apparently the minimum limit has not been determined. When seeds which had been imbibing water for 24 hours were treated longer than five minutes, the abnormalities appeared on the leaves. Table II summarizes the results of three experiments, including a brief description of abnormalities occurring after treatment.

TABLE II

Relation of abnormalities to length of irradiation period. Each experiment was repeated three times and twelve seeds were used in each series

No. weeks after treatment	Time of treatment			
	5 minutes	10 minutes	15 minutes	20 minutes
3	None	First two leaves show mosaic appearance	First two or three leaves like those of mosaic plants*	Severity of injury kills half the plants; abnormality severe even in early stages
4	Very slight abnormalities at tip of leaf	Abnormality comparatively severe and growth of plant irregular	Abnormalities very severe	Abnormalities very severe
5	,,	,,	,,	Growth of plant arrested by injury
6	,,	,,	,,	Very severely injured; plants die later

* One plant had leaves which showed only slight injuries.

The results given in table II show that the minimum limit is less than a 5-minute irradiation period. A further experiment was performed to determine the shortest period of treatment that would produce injury. Five series of plants which were treated 1–5 minutes showed very slight abnormalities at the tips of the leaves in the series treated for four minutes,

four weeks after treatment; the same abnormality was recognized in a series treated for three minutes five weeks after treatment.

Localization of injuries induced by x-rays

Whether the effect of x-rays upon seedlings is localized or not is one of the interesting problems connected with leaf abnormality. Three sets of experiments were performed using seeds which had been imbibing water for 1, 2, and 3 days respectively. No essential difference could be seen in treatment with seedlings of different stages, however, and only a summary of the results will be given.

In a series of experiments, germinated seeds were treated in one set with a metallic screen (3 mm. copper plate) protecting the tops and in another with the screen protecting the roots. The third series was treated without the screen. The fourth, untreated, served as a control. A remarkable difference was observed in the time taken by the seedlings to emerge from the soil. Of the seeds treated for 20 minutes, after they had been planted a week, the control showed 100 per cent. emergence, the top-screened 41 per cent., the root-screened 50 per cent., but none of the unscreened seeds emerged. Generally the root-screened seedlings emerged somewhat more rapidly than the top-screened, while the unscreened were extraordinarily retarded in emergence. Irradiation of the bud retarded growth, so that the control and top-screened seedlings showed almost the same rate of growth; but the elongation of the stem in the other two series was much retarded. The average height of the plants in the latter case is about one-half or one-third of the height of the control plants.

No abnormality appears on the leaf when the top of the plant is protected by the screen; root-screening alone has no effect in preventing appearance of top abnormality, even if the degree of injury is somewhat less than that of the unscreened seeds. The shorter the irradiation period the less the difference of leaf abnormality in the four series (control, top-screened, root-screened, and unscreened).

A complete localization of injuries by x-rays is found in the histological investigation. The incomplete development of vascular bundles in the stem, the abnormality of root cells, and the appearance of dark brown color in the hypocotyl region from the treatment by x-rays, as Johnson (1) reported, were also found and their localization observed in these experiments.

Summary

1. The seed of the sunflower is sensitive to x-rays and often shows abnormal development of vegetative parts on germination after treatment. Abnormality of the leaves is the most common symptom, however, and is constantly induced by a strong irradiation. The leaves become asym-

metrical and crumpled in appearance and develop more or less sinuous contours; they also frequently show irregular coloring of blades. Usually light, dark green, or colorless areas intermingle with ordinary green; even such a peculiar aspect as apparent symptoms of mosaic disease occurs when the injury is severe. Of course, the longer the period of irradiation of the seeds the more marked is the abnormality of the leaf.

2. The internal structure of the colorless part is like that of the ordinary variegated leaf. Cells of palisade tissue are small and slender, frequently curved, and their arrangement is disturbed by the occasional absence of cells.

3. Two kinds of structure are found in light green areas. One is normal as the light color is caused only by the small number of chloroplasts in each cell; the other shows disorder of palisade tissue with much space between cells and an accompanying lack of chloroplasts, which probably have some deficiency in their chlorophyll-absorbing power. Noticeable interference between palisade cells and spongy parenchyma occurs when the entire leaf shows light green color with small streaks of darker green.

4. In the leaves in which dark green tissue is intermingled with normal green tissue no peculiar variations can be found. The deeper color is due only to the greater accumulation of chlorophyll in the chloroplasts.

5. The structural aspect of the leaf with mosaic appearance is more complicated. The four areas, colorless, light green, dark, and normal green, intermingle throughout the leaf structure. The structure, however, is just like that of the preceding color sections except the dark green areas. Strong disturbance takes place in all tissues: the distinction between palisade and parenchyma cells disappears and the chloroplasts aggregate in groups, and sometimes coagulate, giving rise to a bluish green color. Moreover, chlorophyll diffusing out into the cell sap produces a deeper green color.

6. The minimum time limit for inducing leaf abnormality by x-rays is 3 or 4 minutes' irradiation under the following conditions of radiation: 100 peak K.V., 5 ma. current, no screen, 30 cm. focal distance.

7. X-ray injuries seem to be localized and confined to directly treated regions, and to definitely localized cellular tracts. The apparent injury and structural modifications are both localized.

These experiments were performed at the botanical laboratory of the University of Chicago. The writer expresses his thanks to Professor C. A. SHULL for his kindness in suggesting this problem and for his advice in carrying out the experiments.

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OBSERVATIONS ON THE CRACKING OF CHERRIES[1]

Z. I. KERTESZ AND B. R. NEBEL

(WITH ONE FIGURE)

Introduction

The cracking of the fruit is an important problem in most sweet cherry districts. Precipitation during harvest often occasions to growers heavy losses from cracking.

There is little information on this subject in the literature. HARTMAN and BULLIS (**4**) find that in sweet cherries moisture absorption occurs through the roots or through the epidermis of the fruit itself following increased osmotic pressure caused by increases of sugars and soluble solids. VERNER and BLODGETT (**9**) find that cracking in sweet cherries is due to absorption of water through the skin of the fruit. This is influenced directly by the osmotic concentration of the fruit juice, the skin of the fruit, and skin permeability. GARDNER, BRADFORD, and HOOKER (**3**) suggest that elasticity of the skin may be a controlling factor in cracking of fruits.

In the present paper results are reported of cracking tests on cherries of different varieties, together with anatomical and physiological observations. The problem is yet far from an actual solution. It is believed, however, that a few points established and some physico-chemical relations suggested warrant preliminary publication, especially since these studies cannot be continued in the near future.

Morphological considerations

Five cherries from each of seven varieties and from one seedling (Bing, Lambert, Napoleon, Giant, Schmidt, Yellow Spanish, English Morello, and an F_1 seedling, Lambert × Schmidt) were selected and fixed in a weak solution of acetic acid, formalin, and alcohol. Longitudinal strips were cut in such a way that microtome sections could be made equatorially in the neighborhood of the ventral suture. Sections were stained with hematein. Measurements of the epidermal cells were made with an adjustable Spencer ocular micrometer. The subepidermal cell layers were measured by drawing them with a camera lucida upon uniform stock paper, numbering each cell, cutting out approximately 20 cells at a time, and calculating the area from the weight. The cubic content was calculated by multiplying the square root of the square value with the latter, this being admittedly a rough approximation only.

[1] Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper no. 62, December 13, 1934.

An arbitrary distinction is made in that cells from the single surface layer only, covered by the cuticle, were measured as epidermis. All secondary layers of epidermal cells from the outermost one inward to the parenchyma of the flesh proper are called subepidermis. This definition is necessary because, as pointed out by STEVENS (8), it is often impossible to distinguish sharply between epidermis and subepidermis. The size of the epidermal cells in the seven varieties and one seedling used in this study was measured, but no correlation could be found between size of cells and extent of cracking. For instance, English Morello and Bing showed relatively small epidermal cells while Napoleon and Yellow Spanish showed relatively large epidermal cells, yet English Morello and Yellow Spanish resist cracking while Bing and Napoleon crack severely. Furthermore, the sour cherry Montmorency, which does not crack, has epidermal cells similar in size to those of Bing, Lambert, Giant, and Schmidt.

While no correlation could be found between the size of epidermal cells and cracking, an unexplained positive correlation seems to exist between the thickness of the inner wall of the epidermis and the extent of cracking. The results are shown in table I.

TABLE I

RELATIVE THICKNESS OF INNER WALL OF EPIDERMIS OF 8 CHERRY VARIETIES

VARIETY	RELATIVE THICKNESS
Lambert	100
Bing	95
Lambert x Schmidt	95
Napoleon	95
Schmidt	93
Giant	88
Yellow Spanish	78
English Morello	76

Average probable error of mean app. 7 per cent.

The cells of the subepidermis are fairly prismatic in outline. The subepidermis is 3 to 7 cells deep.

Table II shows the outstandingly large subepidermal cell size for Yellow Spanish and English Morello which do not crack ordinarily. It appears that firmness and proneness to crack increase with decreasing size and increasing number of subepidermal cells per unit area. Perhaps this finding is in harmony with DORSEY (2) who noted that the tensile strength of the skin of peaches depends upon the subepidermis or deeper layers which show pronounced lateral stretching during growth.

TABLE II

RELATIVE SIZE OF SUBEPIDERMAL CELLS AND DEPTH OF SUBEPIDERMAL LAYER (AVERAGE FROM 5 CHERRIES)

VARIETY	NO. OF CELLS DRAWN FOR MEASUREMENT	AVERAGE RELATIVE SIZE OF SINGLE CELL	AVERAGE RELATIVE DEPTH OF SUBEPIDERMAL LAYER
Lambert	216	100	100
Schmidt	295	128	105
Giant	224	140	135
Napoleon	163	151	129
Bing	239	162	152
Lambert x Schmidt	218	166	141
Yellow Spanish	218	243	152
English Morello	143	350	252

Average probable error of means ± 10 per cent.

The average depth of the subepidermal layer was found to be greatest in English Morello and least in Lambert (table II). The total absolute depth ranges between 0.125 and 0.25 mm. Compared with the total volume of the flesh of the cherry, this magnitude is small. These findings are in harmony with those of KOSMANOFF (6).

Cross sections of the flesh of sour cherries showed an even distribution of large cells measuring from 200 to 350 μ in diameter. The essential point is that most cells of the flesh proper are of the same size. In the firm-fleshed sweet cherries, on the other hand, groups of large cells are regularly interspersed with groups of cells of $\frac{1}{2}$ or $\frac{1}{3}$ the diameter of the larger ones, the smaller cells measuring only from 50 μ to 150 μ in diameter. While the largest cells of the flesh of firm-fleshed cherries are fully as large as those of the flesh of sour cherries, the average cell size of the firm-fleshed sweet cherry is only about $\frac{1}{2}$ to $\frac{2}{3}$ that of the sour cherry. The vascular bundles are very much more pronounced and cover a much larger area in cross section in the sweet firm-fleshed cherries than in sour cherries.

Physiological considerations

CRACKING OF CHERRIES IN WATER AND IN SUGAR SOLUTIONS

During the season of 1933, ten varieties (Bing, Lambert, Napoleon, Emperor Francis, Giant, Schmidt, Yellow Spanish, Royal Duke, English Morello, and Montmorency) and an F_1 seedling, Lambert × Schmidt, were tested for cracking.

From each of these sorts, 25 selected cherries with the stems attached were submerged for 2, 6, and 18 hours in water and in solutions containing 5, 9.5, 14.1, 18.6, and 23.2 per cent. of sucrose. The cherries were wetted,

TABLE III

SUMMARY OF CRACKING TESTS, 1933*

VARIETY	DATE	AVE. WEIGHT	REFRACTO-METER READING	PER CENT. CRACKING IN WATER IN				PER CENT. INCREASE IN WEIGHT IN WATER IN				REMARKS
				2 HR.	6 HR.	18 HR.	INDEX	2 HR.	6 HR.	18 HR.	INDEX	
		gm.		%	%	%		%	%	%		
Bing	June 22	.....	21.2	0	0	0	0	...	...	.....	.....	
	26	.....	22.9	10	20	80	990	...	...	.....	.....	
	28	6.1	20.0	13	19	38	734	2	5	11	178	
	30	6.3	21.0	0	14	57	481	2	7	12	220	Ripe
Napoleon	June 22	.....	16.8	0	0	0	0	...	...	.....	.....	
	26	.....	18.0	0	0	4	280	...	...	.....	.....	
	28	5.5	19.7	0	0	0	0	...	...	.....	.....	
	30	6.0	17.8	0	24	36	564	2	5	9	172	
	July 3	5.6	21.3	0	32	56	808	1	5	10	155	
	5	5.8	19.7	0	4	52	416	1	5	9	143	
	7	5.8	23.0	0	12	40	436	1	4	8	130	Ripe
	11	5.3	19.3	0	24	76	844	1	4	10	137	
Lambert	June 22	.....	17.8	0	0	0	0	...	...	.....	.....	
	26	.....	18.2	0	20	20	400	...	...	.....	.....	
	28	4.8	15.4	4	4	4	148	3	4	4	128	
	30	5.5	15.0	0	0	0	0	2	5	8	148	
	July 5	6.5	15.2	0	24	88	928	1	5	7	122	
	11	7.6	21.0	0	52	92	1320	2	4	7	143	Ripe
Emp. Francis	July 7	6.7	21.7	0	12	60	576	2	4	9	141	Ripe
	11	6.0	.....	0	12	8	176	2	5	9	163	
Giant	June 30	4.0	15.1	0	0	0	0	1	8	13	217	
	July 5	4.8	15.2	0	0	0	0	1	5	11	176	
	11	5.0	19.5	0	4	24	224	2	5	9	163	
	14	5.2	20.1	0	8	32	436	3	5	8	160	Ripe
Schmidt	June 22	.....	18.8	0	0	0	0	...	...	.....	.....	
	28	4.0	19.8	0	0	0	0	2	4	10	162	
	5	5.1	19.1	0	4	12	136	2	5	9	150	
	7	5.3	20.1	0	0	16	112	2	4	9	144	Ripe
	11	5.3	21.7	0	4	16	164	2	4	8	147	
Yellow Spanish	June 21	.....	17.5	0	0	0	0	...	...	.....	.....	
	26	.....	22.1	0	10	20	270	...	...	.....	.....	Ripe
	28	4.3	22.9	0	0	8	56	2	3	8	124	
Montmorency	July 11	3.6	16.0	0	0	0	0	1	3	4	87	Ripe
	14	3.5	17.0	0	0	0	0	1	2	4	74	

* The results are given in round percentages although decimals were used in calculating the "index" values.

dried, and weighed both before and after submersion. Refractometer readings were made with a Zeiss hand-refractometer on juice expressed from at least 10 cherries. The results of the tests on a few varieties are shown in table III.

Less cracking occurred when the cherries were placed in sugar solutions than in water. No cracking was observed after 18 hours in the 23.2 per cent. sucrose solution. Cracking in most cases increased with progressive ripening. Within any one variety, however, no correlation between the increase in weight of a cherry and cracking could be shown, except in the case of Lambert. No cracking occurred on any cherry lighter than 5 grams in weight. Individual sour cherries weighed approximately 4 grams.

Increase in weight was independent of cracking as expressed by an index of cracking calculated after VERNER and BLODGETT. The index was more than 125 for tests on sweet cherry varieties as compared with an index of less than 88 for samples of Montmorency and 66 for a sample of Royal Duke, both of which showed no cracking. There was a definite water uptake from sugar solutions at all concentrations used.

The refractometer readings on the juice did not show any relation to the cracking of the cherries in different samples as represented by the VERNER and BLODGETT cracking index. Chemical sugar determinations on the juices on which the refractometric readings were taken showed that the readings in the sweet cherries were about 6.5 per cent. higher than the sugar content. HARTMAN and BULLIS state that there is a close relationship between changes in soluble total solids and the changes in total sugars, since the increase in soluble solids is largely an increase in sugars. In the studies of VERNER and BLODGETT the cherries of one variety picked from one tree gave very regular results with regard to increase in total solids, total sugars, and cracking. On the basis of the present results, it is believed that this was a coincidence because both sugar content and cracking commonly increase with advancing maturity.

The increase in weight of the cherries immersed in water showed no correlation to the total solids contained in the juice.

DIFFERENCES IN WATER HOLDING CAPACITY OF THE SWEET AND SOUR CHERRY PULP

Cherries were put through a "Seprosive" which caused thorough crushing of the tissues and also removed the stone and skin by pressing the pulp through a 0.5-mm. sieve. From the resulting mass, which could be well pipetted, 25-cc. samples were placed in wetted folded filter paper inserted into a funnel. The rate with which the liquid passed through the paper was determined by reading at intervals the amount of liquid accumulated

* The results are given in round percentages although decimals were used in calculating the "index" values.

in a graduated cylinder. The results obtained on four varieties of cherries are shown in figure 1.

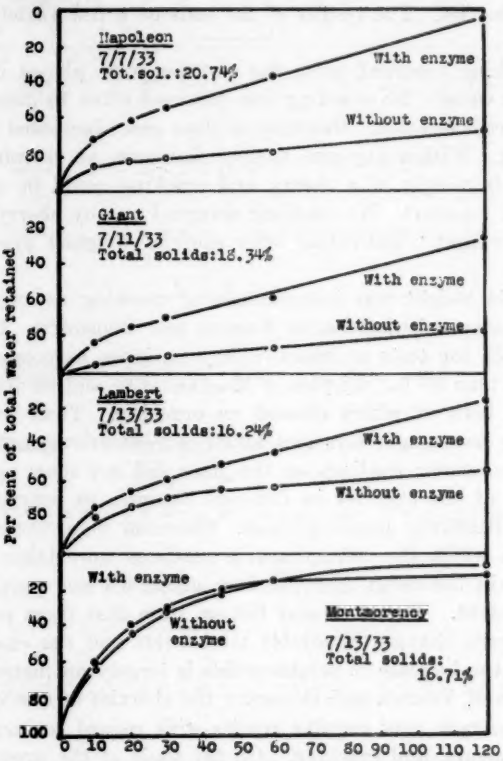

FIG. 1. Water retaining capacity of pulped cherries.

In all of these tests large differences were observed between sweet and sour cherries. As one would expect, the more watery sour cherries after 60 minutes retained only a small fraction of their original water content while the more fleshy sweet cherries yielded barely $\frac{1}{3}$ of their original water content.

Further experimental evidence shows that the difference observed was caused by the colloids present. The 25-gram samples of the pulped cherries were treated with 0.5 gram of a mixture of highly active pectic, amylolytic, and proteolytic enzymes for 30 minutes and the mixture poured on to the filter. The water-retaining capacity of the sour cherries, as indicated by the results obtained with Montmorency, showed no significant difference after enzyme treatment. The pulp of the sweet cherries showed a marked

change, however, inasmuch as it filtered much faster than without the enzyme treatment. In a few cases, after longer than 30 minutes of treatment by the enzyme, the water passed through at a rate almost equal to that of the sour cherries.

The importance of these experiments lies in the fact that they show to what a great extent the water-holding capacity is influenced by the colloids present. They prove that the great difference in the water-holding capacity of sweet and sour cherries with only a slightly different content of total solids is caused by the colloids of the sweet cherries.

Some work has been done by the senior author on the differences in the chemical composition between sweet and sour cherries, especially as regards sugar and pectins, which shows that both of these constituents are present in a lower percentage in sour cherries. These results, however, are determinations of a single date only and would mean more if progressive changes in the cherries in several of these constituents were followed throughout the growing season.

Discussion

The morphological studies showed a difference in the subepidermis of cracking and non-cracking cherries. The fact that non-cracking cherries showed relatively large cells in the subepidermis does not explain the phenomenon of non-cracking mechanically. A structure built of smaller units would be expected to have relatively higher tensile strength. The skin (epidermis and subepidermis) of firm-fleshed sweet cherries is probably stronger than that of soft-fleshed sweet and sour cherries, but not sufficiently so to overbalance the relatively higher expansion force of the flesh of the former. In this case also a physical study of this factor should be made upon the isolated tissue but with consideration of the natural forces acting on it *in situ*.

A search of the horticultural literature fails to give any useful information on the permeability of the skin of cherries to water. The morphological studies here reported fail to indicate any difference in the structure which might have a bearing on permeability.

At present it is considered hazardous to correlate cracking in cherries with morphological findings beyond the distinctions which separate firm- and soft-fleshed groups of varieties which, for practical purposes, are obvious without the use of a microtome.

So far as the writers are aware, the swelling of cherries when immersed in water has been attributed solely to osmotic absorption of water through the skin of the fruit. It is stated by VERNER and BLODGETT, and by HARTMAN and BULLIS, that the highest sugar content of the cherries was not always associated with the most severe cracking. The discrepancy in

this respect is most apparent at full maturity. The changed turgor is said to be responsible for this phenomenon. It is most plausible to presume, however, that absorption by the flesh of cherries and probably also turgor in general is only partly caused by osmosis. The swelling of the colloids present in plant tissues may be responsible for a certain part, if not the major part, of the turgor. The enlargement of certain plant parts, especially of fruits (in a broader sense), is caused mostly by the formation of succulent plant tissues which carry at any time a definite proportion of water. At least a part of the materials present are formed in the fruit itself, causing considerable changes in the water requirements of the tissue at the time of their formation.

It has been suggested that the different behavior of the colloids of sweet and sour cherries might be the result of diversities in their actual acidity. The studies of CALDWELL (1), however, showed that there is not much difference between the hydrion concentration (pH) of sour and sweet cherries. It is more likely that the qualitative and quantitative differences in the colloids are responsible for the differences in cracking.

The water-holding capacity of any plant or plant tissue is, under normal conditions, only partly satisfied. This saturation deficit is indicated by the quick swelling of plant materials not only in water but also in solutions of considerable concentrations (ILJIN 5). This phenomenon can be observed on all varieties of cherries at any stage of maturity because they all take up water when immersed in it.

There are reports in the literature on the high pressures exerted by swelling colloids. Swelling starch, for instance, can produce a pressure of over 2000 atmospheres (KOSTYCHEV 7) and many other colloids show a similar behavior.

It is considered likely that attraction for water by the cherry fruit is caused by the constituents of the cell walls. As the morphological studies have shown, these materials are present in larger quantity in the firm-fleshed sweet cherries. Since the sugar content of firm sweet, soft sweet, and sour cherries is not appreciably different, and since the attraction of the pulp for water does vary, this variation is held to be due to differences in the colloid content of the fruit and is considered responsible for the cracking of cherries.

Summary

1. The size of the subepidermal cells in cherries which are prone to crack is appreciably smaller than in those which do not crack, the number of subepidermal cells per area being correspondingly larger.

2. In cherries which tend to crack, the large parenchymatous cells of the flesh are more frequently interspersed with smaller cells, thus decreas-

ing the average cell size considerably. In the non-cracking types examined, the average cell size of the flesh is relatively larger and more uniform.

3. The size of cells of the epidermis is not correlated with proneness to crack. Thickness of the inner wall of the epidermis is positively correlated with cracking.

4. Both the rate and extent of cracking of cherries are lessened when the fruits are immersed in sugar solutions instead of in water. No cracking could be observed on cherries immersed in 23.2 per cent. sucrose solution, and only 19 per cent. of the samples which cracked in water cracked when immersed in 18.6 per cent. sucrose solution for 18 hours.

5. All cherries used in this study took up water when immersed in water or in sucrose solutions. Although no correlation between water uptake and cracking could be shown within one variety, the cracking and non-cracking cherries formed two distinctly separate groups, the water uptake in the latter group being much lower. This fact indicates a difference in saturation deficiency of the cherries of the two groups.

6. It is suggested that the cracking of cherries is caused by the forces of the swelling colloids of the flesh rather than by osmotic forces.

7. In the foregoing statements, "cherries prone to crack" is roughly equivalent to hard-fleshed varieties, "cherries not prone to crack" grossly corresponds to soft-fleshed varieties.

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STREAMLINE FLOW AND THE MOVEMENT OF SOLUTES IN THE TRANSPIRATION STREAM

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Introduction

The classic experiment of Askenasy (**1, 2**), illustrating the pull exercised by an evaporating water surface, consists, as is well known, of a thistle funnel closed at the wide end by a plug of plaster of paris, made airtight, if necessary, by ringing with gold-size. The funnel is filled with well boiled distilled water, and inverted into a vessel of mercury. As the water evaporates from the upper surface of the plaster plug the mercury rises *pari passu* at the lower end of the tube, and might be expected to reach eventually barometric height, less the vapor pressure at the prevailing temperature. Actually the surface tension and the molecular cohesion of the water draw the mercury to much greater heights. Askenasy observed a rise of 89 cm. at a barometric pressure of 73.5 cm. Ursprung, in a series of papers (**7, 8, 9, 10, 11**), made more extensive observations and obtained a rise of 135 cm. above barometric height, in glass tubes, and of double barometric height in liane stems used as tubes. In all cases, bubbles are formed eventually and the cohesion breaks down.

The rate at which the mercury rises depends, of course, primarily on the rate of evaporation and secondarily on the ratio between the area of the evaporating disc and the cross section of the tube. The rate, however, is not exactly equivalent to the rate of removal of the water. Nordhausen (**3**) has shown that an evaporating surface can raise water from a vacuum, *i.e.*, develops beneath it a negative pressure of at least one atmosphere. Our own experimental comparisons of the weight/volume ratio during the rise of the mercury column showed a diminution in the density of the water of as much as 10 per cent., so that as the experiment proceeds an increasing negative pressure develops in the water, long before barometric height is reached, which tends to withdraw the water menisci from the pores of the evaporating surface and thus check the rate of flow.

The existence of this negative pressure can readily be shown by using a thistle funnel with plaster plug, the stem of which dips into mercury in a closed filter flask. The flask is partly evacuated and time is then allowed for a negative pressure to establish itself and for the mercury to rise a short distance in the stem of the funnel. On admitting air to the flask the mercury rises with a sudden bound upward, often to twice its previous height, in replacement of the contracted water column.

Procedure

If, during the course of the ASKENASY experiment, a small amount of a strongly colored solution is introduced at the bottom of the tube by means of a curved pipette, so that the solution floats above the mercury, it will be observed that the upper surface of the colored layer does not rise as a level surface, but is drawn up in a delicate axial thread of color which advances at a surprising speed and reaches the top of the tube in a few minutes, even at low rates of evaporation. An array of dyes was tested in this way and it was found that the rate of rise was independent of the molecular weight of the dye used, and is therefore not a simple diffusion phenomenon. Moreover, the characteristic movement is absent in tubes sealed at the top.

For our experiments the original arrangement of the apparatus was modified only by the addition of an adiathermic shield, formed of a wide glass sleeve filled with a saturated solution of nickel sulphate. As each funnel has its own characteristics, compared tests were always made with the same funnel.

Observations

The apex of the column is sharply defined and usually obtuse. The latter point is striking. The appearance is different from that which one sees on applying a hydrostatic suction to the upper end of the water column when the color is drawn up axially in fine vanishing point. In this latter case, neutral red introduced over a 2-inch column of mercury forms a dense layer, with no streaming, until gentle suction is applied by opening a capillary siphon attached to the top of the tube. As soon as flow starts the color is immediately drawn out along the median line, forming a narrow cone with straight sides, which lengthens out so long as flow continues. When the siphon is closed the flow stops and the color sinks slowly back by gravitation into a uniform diffusion gradient. Left thus undisturbed the color took two weeks to reach the top of a 2-foot tube.

The rates of rise observed for the axial column of color are given in table I.

The rates of flow are linear until the top of the tube is reached, irrespective of the dye used.

The air temperature of the laboratory varied throughout the experiments, but the mean deviation of the averages for each group of tests from the average for the whole series was only 1.0° C., so that temperature changes cannot account for the observed differences.

Although experiments with sealed tubes and siphons, referred to above, show that the phenomenon is not one of diffusion pure and simple, there is a general relation between the diffusibility of the dyes and the rate of

TABLE I

RISE OF DYES IN ASKENASY TUBES*

(AVERAGES OF THREE OBSERVATIONS IN EACH CASE)

DYE USED	MOLECULAR WEIGHT	AVERAGE TIME TO RISE 10 INCHES (HALF LENGTH OF TUBE)
Thionin	263	24 minutes 31 seconds
Neutral red chloride	288	12 minutes 53 seconds
Methylene blue	319	10 minutes 47 seconds
Safranin	350	6 minutes 25 seconds
Bismarck brown	418	3 hours to 6 inches only
Janus green B	422	20 minutes 39 seconds
Methyl violet 6B	482	14 minutes 45 seconds
Benzopurpurin	628	No rise
Congo red	696	42 minutes to 4 inches only
Trypan red	766	60 minutes 0 seconds
Trypan blue	966	No rise

* Height of mercury in each case before introduction of dye, 1.4 inches.
Height of mercury after introduction of dye, 1 inch.
Quantity of dye (0.5 per cent. solution) introduced, 0.3 cc.
Mean air temperature, 18.4° C.

rise. Some of the dyes used are colloidal or semi-colloidal, and their solution aggregates are apparently too heavy to be raised in the flow lines of these tubes. Such are benzopurpurin, congo red, and trypan blue, as table II shows.

Theoretical considerations

The comparative rate of rise, however, is only of secondary importance in consideration of the remarkably rapid movement of practically all the

TABLE II

COMPARISON OF RATE OF RISE AND DIFFUSIBILITY

DYES IN ORDER OF RATE OF RISE	ORDER OF DIFFUSIBILITY INTO 10 PER CENT. GELATIN
1. Safranin	1
2. Methylene blue	2
3. Neutral red chloride	4
4. Methyl violet	6
5. Janus green	5
6. Thionin	3
7. Trypan red	8
8. Bismarck brown	7
9. Congo red	10
10. Trypan blue	9
11. Benzopurpurin	11

dyes, in the axial line. This appears to be due to the actual vertical flow of the water column and finds its explanation in the classic researches of REYNOLDS (5) on water flow in parallel-walled channels.

In REYNOLDS' experiments the colored fluid was introduced into the water current from a fine jet near the inlet end of the flow tube. At low speeds the color was drawn out into a fine axial thread, but as the rate of water flow increased the thread showed disturbance, due to eddies, near the outlet. This turbulent flow gradually approached nearer to the inlet end, until at a given speed the whole flow became turbulent. If the rate of flow was again reduced, it was found that the turbulent flow again gave place to linear flow at a fairly definite velocity, called the "critical velocity."

No sign of turbulent flow was observed in our experiments. This was according to expectation, for the flow due to evaporation is extremely slow and well below the critical velocity.

We may conclude that the flow of water in xylem vessels, in common with that in the experimental tubes, is purely of streamline type. The following considerations will make this clear.

In streamline flow, the inertia forces are small compared with the tangential frictional forces between neighboring layers of fluid. Calculations of the rate of flow, based purely on viscosity, agree so well with experimental observation that the reverse calculation is used as a standard method for measuring viscosity. When a fluid is moving over a smooth plane surface, the layer actually in contact with the surface is at rest, and each successive layer outward moves with greater velocity, *i.e.*, there is a velocity gradient normal to the surface. If v is the velocity parallel to the surface at any distance, y, measured perpendicular to the surface, then the velocity gradient at the surface is given by $\frac{dv}{dy}$.

Applying this to flow in pipes, REYNOLDS found that the critical velocity, v_c, occurs under all conditions at a constant value of the ratio $\frac{v_c \delta \rho}{\mu}$, where δ = the diameter of the pipe, ρ = the density of the fluid, and μ = the viscosity coefficient. This ratio has come to be known as the REYNOLDS number. It is valid for all conditions of flow and with all fluids, and its average value is 2000. As a ratio it is non-dimensional.

The mean critical velocity for water is therefore $2000 \times \frac{\mu}{\delta \rho} = \frac{26}{\delta}$ at 15° C., where δ is the diameter of the pipe in centimeters. The critical velocity is therefore inversely related to the diameter of the tube. Assuming a diameter of 1.0 mm. for a xylem vessel, as an extreme case, the average critical velocity for water at 15° C. in such a tube would be 2.6 meters per second, which is well above physiological limits.

If, therefore, we are entitled to assume a condition of streamline flow as existing normally in xylem channels, then the difference between the axial and the average velocities in the moving columns of water in the vessels becomes a matter of considerable physiological importance.

OWER (4), discussing REYNOLDS' work, points out the conditions for the determination of this difference. Consider any cylindrical element of radius r, assumed to lie in the axis of the pipe, the upstream end being denoted by A and the downstream end by B, the distance between them being dl. The pressure on A = p and on B = p − dp. The case is the same whether a positive pressure be applied to A or a negative pressure to B. The pressure difference maintaining the motion of AB is then $\pi r^2 dp$. This quantity must equal the retarding force of fluid viscosity acting on the exterior of the cylinder, that is to say $2\pi r dl\mu \frac{dv}{dr}$, when v = the velocity of flow at radius r.

Therefore

$$\pi r^2 dp = -2\pi r dl\mu \frac{dv}{dr} \quad \ldots\ldots (1)$$

and

$$\frac{dp}{dl} = -\frac{2\mu}{r}\frac{dv}{dr} \quad \ldots\ldots (2)$$

As the pressure does not vary across the section of the cylinder, $\frac{dp}{dl}$ is constant for given conditions, and as velocity in streamline flow varies only with r, then $\frac{2\mu}{r}\frac{dv}{dr}$ is a function of r only.

Now $\frac{dp}{dl}$ may be written as equal to $\frac{p_1 - p_2}{l}$ where p_1 and p_2 are the respective pressures at each end of l.

Therefore $\frac{p_1 - p_2}{l} \cdot r dr = -2\mu dv$ ..... (3)

Integrated from r = 0 to r = R the full radius of the tube, $\frac{p_1 - p_2}{l}\frac{2}{R^2} = -2\mu(V_R - V_a)$, when V_a = the velocity at the axis, and, as the velocity at the wall of the tube is zero, $V_R = 0$ and

$$\frac{p_1 - p_2}{l} = \frac{4\mu V_a}{R^2} \quad \ldots\ldots (4)$$

Thus

$$V_r = \frac{p_1 - p_2}{4\mu l}(R^2 - r^2) \quad \ldots\ldots (5)$$

which shows that the distribution of velocity according to radius is parabolic across the tube section.

Also, from equation (4) we have

$$V_a = \frac{p_1 - p_2}{4 \mu l} \cdot R^2 \quad \text{.....(6)}$$

From the classic formula for absolute viscosity: Volume flowing in unit time $= \frac{\pi R^4 (p_1 - p_2)}{8 \mu l}$ and volume flowing in unit time also $= \pi R^2 V_m$ where V_m is the mean velocity of flow.

Therefore the mean velocity, $V_m = \frac{p_1 - p_2}{8 \mu l} R^2$ ..... (7)

so that the axial velocity (equation 6) is twice the mean velocity.

Even where the absolute amount of flow is slight, there will be a relatively rapid movement of the axial column of liquid, as is evidenced by the rapid ascent of the dye solution in our experiments. Comparison of observations, made with the same apparatus, of the halfway rise of neutral red chloride at varying rates of evaporation, as measured by the number of minutes taken by the mercury to rise from zero to 1 inch, show, however, that the relationship is not so simple as the preceding reasoning suggests. According to equation (6) the relationship of axial velocity to pressure in the water column is directly linear. When the times of rise are plotted against rates of evaporation, however, the points show, although with considerable scatter, a convex distribution with a mode near the 1000-minute line, under the conditions of the experiment. On either side of this line the rate of dye ascent increases, with the paradoxical result that increasing the rate of evaporation from say 2000 to 1000 minutes per inch of mercury may have the effect of decreasing the rate of dye ascent, and vice versa. Any movement toward the mode, from either direction, is a move toward slower rates of ascent.

It is also found that extreme reduction in the rate of evaporation, short of absolute stoppage, has comparatively little effect on the rate of dye ascent, although it reduces the amount of dye raised. Thus, in one case where the mercury took 8 days (11,150 minutes) to rise from 0 to 1 inch, neutral red, when introduced at the end of this period, rose 10 inches in 10 minutes and 30 seconds.

In the other direction, the rate of ascent increases rapidly as the evaporation increases, until a point is reached, not very exactly defined but in the region of the 200-minute rate, where the phenomenon appears to cease altogether, although the rate of water movement is still considerably below the critical velocity. This result, at first sight surprising, may be deduced from the consideration of equations (5) and (6).

From equation (5) it will be seen that as $p_1 - p_2$ increases V_r will increase toward the limiting case, determined by the REYNOLDS number, where streamline flow ceases. At this point $v_r = v_a$ and the radial gradient of velocity disappears. As this condition is approached r^2 diminishes and r

therefore becomes vanishingly small some time before the limit is reached; that is to say, the axial movement is restricted to a line of water too tenuous to draw up any observable amount of dye. The surface of the dye layer will therefore appear to remain undisturbed until true turbulent flow sets in. This corresponds presumably to the region of instability found by STANTON and PANNELL (**6**).

Conversely it may be seen that as $p_1 - p_2$ diminishes, with lower rates of evaporation, the consequent diminution of v_r may be compensated by the diminution of r^2. Reference to equation (1) shows that r is a more important factor in the equilibrium of force against viscosity than v, the velocity, and that equilibrium will be more economically maintained by changes of r than of v. With diminishing r one might therefore expect v to be maintained down to low values of $p_1 - p_2$.

A point of particular interest is that, in the evaporating system of the ASKENASY tube, there may be a movement of material against a concentration gradient. If a small quantity of dye be introduced at the base of the tube the axial current will shortly draw it all to the top of the tube, where it accumulates, so that the latter fractions of the dye material are moving toward a region of concentration.

Discussion

The application of these ideas to the conditions of water flow in the xylem looks, in the first place, to the cases where, under conditions of very low transpiration, such as obtain in very humid and sometimes also in very dry situations, there is little or no negative pressure and the cell elements are full of water. In any such case, short of complete hydrostasis, solutes will travel up the axes of the water channels at speeds considerably greater than the total measured water flow would suggest.

Even where air bubbles are present in the vessels, the free water-air surface will still travel at a rate at least twice the mean rate of flow, which will itself be locally accelerated in such conditions by the restriction of the available channel.

Observations at reduced rates of evaporation suggest that under such circumstances a tension may develop which leads to axial rates in excess of twice the mean velocity. In the case just mentioned, for example, where mercury took 8 days to rise 1 inch, the dye rose 10 inches in $10\frac{1}{2}$ minutes. Observations made under constant conditions of evaporation, but starting with the mercury at varying heights in the tube, also show a consistent increase of velocity of axial flow as the mercury level rises.

The rate of flow is also slightly greater, under all conditions, in the upper part of the tube, but most markedly so when evaporation is reduced, that is to say under those circumstances where local tension is most likely to develop. The state of tension, most accentuated at the axis, reduces the

density and hence the viscosity, to which the velocity of flow is inversely related.

Summary

The problem of the diffusion of solutes in plants with very low rates of transpiration may be explained by consideration of the radial velocity gradient due to streamline flow in tubes.

With low rates of flow, there is a parabolic velocity gradient normal to the walls of the tube, and an axial current of at least twice the mean velocity of flow. Experiment shows that these conditions are maintained, and even exaggerated at very low rates of water movement, and only disappear, in the one direction at complete hydrostasis and in the other direction at a velocity some way short of the beginning of true turbulent flow, which latter cannot, however, normally occur in the transpiration channels. Movement of the solute under these conditions may take place toward a region of concentration.

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UNFREEZABLE AND FREEZABLE WATER EQUILIBRIUM IN PLANT TISSUES AS INFLUENCED BY SUB-ZERO TEMPERATURES[1]

GLENN A. GREATHOUSE

Introduction

It is commonly accepted that with biocolloidal systems there is a definite temperature at which all of the freezable water will be frozen (**7, 11, 6,** and others). This temperature has been arbitrarily selected as about – 18° to – 20° C. In studying one phase of a problem on winter hardiness in red clover roots, it was desirable to determine the amount of unfreezable water in these tissues. Thus an opportunity was afforded to test the preceding hypothesis with regard to this tissue by determining the amount of water which does not freeze at temperatures of – 10° to – 50° C. Potato tubers were also included in the study.

Review of literature

ST. JOHN (**9**) made a study of the unfreezable and freezable water equilibrium of the thick portion of egg white, employing the method of RUBNER (**7**) as modified by THOENES (**11**) and ROBINSON (**6**). He found that at – 5° C., 80 per cent. of the water present was unfreezable, while at – 10° C. only 35 per cent. remained unfrozen. Determinations at – 12.5° C. showed 25.8 per cent. of the water unfreezable. From this temperature on through – 35° C. he found a flat straight-line curve indicating that no more of the water was frozen at a temperature of – 35° C. than at – 12.5° C.

JONES and GORTNER (**3**), using the dilatometer method on gelatin solutions, found that all of the freezable water was crystallized out at – 6° and no additional amount was removed at a temperature of – 50° C. With a 2 per cent. solution the percentage of total water unfreezable was 9.35. At a temperature of – 44.4° C. the unfreezable water percentage was exactly the same as at – 6° C. They state that animal and plant tissues and related systems would probably behave like the gelatin system. It does not necessarily follow, however, that such would be the case for plant tissues.

JONES and GORTNER's (**3**) data on inorganic hydrogels indicate that there was no temperature within the range of 0.0° to – 50° C. at which a lower temperature did not cause the crystallization of additional quantities of ice.

[1] The writer expresses his appreciation to Dr. E. A. HOLLOWELL, of the Forage Crop Division, U. S. D. A., for furnishing the clover seed used in this investigation and for his valuable suggestions in culturing red clover plants.

Methods

A variety of methods have been used by investigators for determining the ratio of unfreezable to freezable water in organic materials. Of these the early method of MÜLLER-THURGAU (5), later modified by RUBNER (7), THOENES (11), ROBINSON (6), ST.JOHN (9), SAYRE (8), and MEYER (4), was chosen. This is the calorimetric or heat of fusion method. It has been modified slightly to meet the requirements of this particular problem. The symbols and equation used follow in general those developed by previous investigations.

The tissues used in the unfreezable water determinations were put through a Nixtamal mill. Portions were then taken for total and freezable water determinations, the total moisture being determined in a vacuum oven at 80° C. and 3 to 4 cm. pressure. The total moisture value was used in the unfreezable water calculations instead of the moisture determinations on the same following the calorimetric determinations, as has been done by a number of previous investigators.

For the freezable water determination, 8 to 10 gm. of tissue were placed in a tared tinfoil cup, similar in construction to that used by ROBINSON (6), which was then placed in a tared weighing bottle. The whole was weighed accurately and placed in the low temperature cabinet at the desired sub-zero temperature. For the temperatures of $-10°$ to $-23°$ C. a specially constructed cabinet was used. For temperatures of $-25°$ to $-50°$ C. an alcohol bath, to which dry-ice was added to give the desired low temperature, was employed. The inner container of tinfoil is essential in weighing the tissue and in making a quick transfer of the frozen tissue to the calorimeter. It likewise aids in preventing (1) floating of tissue on surface of water in calorimeter, (2) evaporation of water from tissue during weighing, etc., and (3) heat of solution of any soluble substances while in the calorimeter. The outer container aids in the identification and transfer of the tissue from the sub-zero temperature to the calorimeter with the minimum of time and exposure to room temperature.

The determination of unfreezable water by the calorimetric method requires the establishment of a stable phase equilibrium at the desired sub-zero temperature. The value for unfreezable water varies with temperature, within limits, and it is important to maintain the desired freezing temperature constant, preferably with $\pm 0.25°$ C., for a period of hours. The requirement of $-10°$ to $-23° \pm 0.25°$ C. was fulfilled in a special low temperature unit constructed by the Freas Electric Company.

The freezable water samples were allowed to remain in the cabinet for 10 to 12 hours. Triplicate samples with different time intervals indicated that the different phases had attained an equilibrium in this period.

The calorimeter consisted of a highly evacuated silvered Dewar flask, closed with a cork 3 cm. thick. Two holes were placed through the cork, one for the thermopile or Beckmann thermometer and the other for a spiral glass stirrer. Proper stirring of the liquid is essential to establish an equilibrium between the material and the liquid of the calorimeter.

Exactly 300 gm. of distilled water were placed in the calorimeter. As soon as the water had come to a temperature equilibrium with the calorimeter walls, glass stirrer, and thermo-elements, the frozen sample was introduced and the new equilibrium determined. The time required to reach equilibrium for the 8 to 10-gm. sample was 8 to 10 minutes, with a constant rate of stirring. The temperature was measured by a Beckmann thermometer or by the use of a 15-junction thermopile attached to a Leeds and Northrup type K potentiometer. The thermopile was constructed of 28 gauge copper and constantan wire. It was possible with this thermopile and accessory equipment to measure 0.001° C.

With the addition of the frozen sample to the water of the calorimeter there is a fall in temperature. In this investigation the heat lost by the water in the calorimeter is used in warming six different component parts of the system. Each of these quantities may be expressed as follows:

$$(1)\ W_t\ S_t\ (T_e - T_s)$$
$$(2)\ W_d\ S_d\ (T_e - T_s)$$
$$(3)\ W_b\ S_b\ (T_\triangle - T_s)$$
$$(4)\ W_i\ S_i\ (T_\triangle - T_s)$$
$$(5)\ W_w\ S_w\ (T_e - T_\triangle)$$
$$(6)\ H_f\ W_i$$

W_t = weight of tinfoil cup;
W_d = weight of dry matter in sample;
W_b = weight of unfreezable water in sample;
W_i = weight of ice in sample;
W_w = total water in sample;
S_t = mean specific heat of tinfoil for temperature range used;
S_d = mean specific heat of dry matter for temperature range used;
S_b = mean specific heat of unfreezable water for temperature range used;
S_i = mean specific heat of ice for temperature range used;
S_w = mean specific heat of water for temperature range used;
H_f = heat of fusion of ice (79.75 calories) at $T_\triangle$;
T_e = equilibrium temperature;
T_s = sub-zero temperature of sample;
$T_\triangle$ = freezing-point of water in plant sap.

It is necessary to determine a correction factor for the calorimeter, since the walls of the calorimeter, stirrer, etc., absorb heat from the water the same as the components in the system. This correction factor was obtained by placing 8–10 gm. of water in the tinfoil cups, freezing at the desired temperature, and then transferring to the water of the calorimeter and determining how much heat was necessary to melt the ice and raise the temperature of the resulting water to the equilibrium temperature of the system. The values for the correction factor were plotted for the different sized samples. Thus the factor could easily be obtained for the sample that varied from 8 to 10 gm. For an 8-gm. sample the correction factor was found to be 1.085.

It is evident that the loss of heat energy in terms of calories by the water in the calorimeter may be expressed mathematically as follows: (7) $FNS_w(T_o - T_e)$

where F = calorimetric correction factor;

N = number of grams of water in calorimeter (300 gm.);

S_w = specific heat of water for temperature range used $(T_o - T_e)$;

T_o and T_e = original temperature of water and equilibrium temperature respectively.

Specific heat determinations were made on the dried tissue, following the procedure previously outlined, except that benzene was substituted for the water in the calorimeter. It was found that the use of a liquid of lower specific heat increased the accuracy of the determination. The values used for the specific heat of pure benzene were secured by plotting a curve for the values as given in the International Critical Tables (**12**).

The specific heat values for ice were taken from DICKINSON and OSBORNE (**1**). The values for water above zero were taken from the Handbook of Chemistry and Physics (**2**). The specific heat values for water below zero were obtained by extending the curve of values from 0° to 30° C. as a straight line, assuming that the specific heat of water continues as a linear function, as has previously been done by SAYRE (**8**). It is assumed that the specific heat of unfreezable water is the same as that of freezable water at the same temperature.

Combining quantities 1, 2, 3, 4, 5, 6, and 7, equating and solving for W_1, the final complete formula becomes:

$$W_1 = \frac{FNS_w(T_o - T_e) - W_t S_t(T_e - T_s) + W_d S_d(T_e - T_s) + W_w S_w(T_e - T_s)}{H_f - (S_b - S_1)(T_\triangle - T_s)}$$

The values for T_s and $T_\triangle$, being below zero, are indicated in the formula by a negative sign. The unfreezable water is determined by subtracting the freezable water from the total water, thus $W_b = W_w - W_1$.

Results

The temperatures used were from – 10° to – 50° C. by five degree steps. In addition measurements were made at – 22° C. The physical chemist (**10**) considers – 22° C. as the minimum freezing-point of pure water under any set of equilibrium conditions. The data presented in table I show the variation in results for typical triplicate samples and indicate the accuracy of the method.

TABLE I

PERCENTAGES OF UNFREEZABLE WATER IN UNHARDENED CLOVERS AT DIFFERENT SUB-ZERO TEMPERATURES (OHIO VARIETY)

SUB-ZERO TEMPERATURE °C. ± 0.25	UNFREEZABLE WATER	
	TOTAL WATER	TOTAL SOLIDS
	%	%
– 10	20.502	103.936
	20.548	103.988
	20.536	103.926
– 15	12.011	60.786
	13.940	70.543
	14.501	73.387
– 20	8.672	43.883
	9.555	48.353
	9.307	47.101
– 22	7.057	35.716
	7.925	40.106
	7.279	36.853

Table II presents representative data that have been calculated from measurements made upon clover and potato tissue when exposed to temperatures from – 10° to – 50°. It will be noted that as the temperature decreases from – 10° to – 50° C. (table II, A and C) in the case of the unhardened red clover root tissue, the unfreezable water value also decreases. A similar condition can be observed on the hardened-off clover root tissue between – 15° and – 20° C.; however, from – 20° to – 25° C. the unfreezable water value remains nearly constant. Part C of table II presents data to show the influence of very low temperatures upon the unfreezable water values. It will be noted that there is a lowering of the unfreezable water fraction with each five degree fall in temperature. This is especially marked between the temperatures of – 10° to – 30° C. The rate of decrease becomes slower with the lower sub-zero temperatures. Potato tissue (table II) behaves similarly to clover root tissue, with the exception that the unfreezable water ratio is shifted slightly farther to the right.

TABLE II

INFLUENCE OF SUB-ZERO TEMPERATURES ON PERCENTAGE OF UNFREEZABLE WATER IN CLOVER AND POTATO TISSUE

KIND OF TISSUE	SUB-ZERO TEMPERATURE °C. ± 0.25	UNFREEZABLE WATER		TOTAL MOISTURE
		TOTAL	TOTAL SOLIDS	
		%	%	%
RED CLOVER ROOTS— A: Ohio, unhardened, same age as B	−10	20.53	103.95	83.50
	−15	13.48	68.24	
	−20	9.18	46.45	
	−22	7.42	37.56	
B: Ohio, hardened, same age as A	−15	15.43	78.06	82.30
	−20	13.16	68.17	
	−22	14.03	71.04	
	−25	13.65	69.09	
C: Unknown variety grown in field, unhardened	−10	14.47	72.16	81.81
	−15	9.43	52.04	
	−20	7.36	38.66	
	−22	5.28	24.73	
	−25	2.66	14.23	
	−30	1.99	11.76	
	−35	2.11	12.84	
	−40	1.95	11.54	
	−45	1.50	10.63	
	−50	1.43	10.59	
D: Potato (Irish Cobbler) tubers	−10	11.05	47.31	81.07
	−15	9.16	38.18	
	−20	7.75	33.18	
	−23	5.59	23.96	

Discussion

A number of investigators have suggested that water binding in biological materials follows an adsorption reaction. The results of this investigation indicate more nearly an equilibrium between the external force (low temperature) and internal forces of the tissue.

The writer's experimental data with plant tissues exposed to sub-zero temperatures do not follow exactly the generalizations of JONES and GORTNER (3) on gelatin, or that of ST.JOHN (9) on egg white. The results of table II, parts A, C, and D, follow more closely the data that have been secured by different investigators on inorganic hydrogels; whereas the data in table II, B, more nearly coincide with the findings of JONES and GORTNER on gelatin and ST.JOHN on egg white. However, the constancy of unfreezable water values occurs at lower sub-zero temperature.

There are indications that the same tissue (table II, A and B), when grown under different conditions, as temperature, will produce entirely different values for the unfreezable water value when the determinations are made at −22° or −25° C.

Summary and conclusions

1. The hypothesis of RUBNER (7), THOENES (11), ROBINSON (6), and others that biocolloidal systems have a definite temperature (– 18° to – 20° C.) at which all of the unfreezable water will be frozen has been tested for plant tissues. This hypothesis is not supported by unhardened clover roots, but seemed to be by the hardened clover root tissue. The unfreezable water values decreased 1.40 per cent. for the cold hardened red clover roots with the lowering of the temperature from – 15° to – 22° C., whereas the unfreezable water values of the unhardened root tissue decreased 6.02 per cent. over the same temperature range.

2. With another lot of unhardened red clover root tissue there was a decrease in the amount of the unfreezable water expressed as the percentage of the total water, from 14.47 to 1.43 per cent. with the decrease of the temperature of – 10° to – 50° C.

3. Potato tissue gave similar results to those of unhardened clover root tissue, with the exception that the unfreezable water values were lower for similar sub-zero temperatures.

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HORMONES IN RELATION TO ROOT FORMATION ON STEM CUTTINGS[1]

WILLIAM C. COOPER

(WITH FOUR FIGURES)

Experimental work with one of the root-forming hormones, β-indolyl-acetic acid, has for the most part been limited to root formation on pea cuttings. This paper presents results obtained from applying the hormone to stem cuttings of other plants with the purpose of determining its practical value in propagation by cuttings.

Pure synthetic β-indolyl-acetic acid was used in all of the experiments and was applied to the cuttings by the lanolin method of LAIBACH (**2**). One part of the hormone was mixed with 2000 parts of pure lanolin, in which it is soluble. A small portion of this paste—roughly about 10 mg.—then was smeared on a small area, about 0.5 × 0.125 inch, on one side of the cutting near the top which had previously been scraped to remove the epidermis and outer cortical layers.

Lemon experiments

Twigs of mature wood of the current and of the previous year's growth were taken from a Eureka lemon tree on April 30, 1935, and were made into 80 cuttings about 5 inches long and with 4 or 5 leaves. The cuttings were divided into 10 lots and treated as shown in table I. Girdling, when used, consisted of removing a complete ring of bark about 1 cm. in width at about 1.5 in. from the base of the cutting. All leaves below the girdle were removed and, in the case of the treated cuttings, the hormone was applied above the girdle. Only the portion of the cutting below the girdle was placed in the sand.

The results presented in table I and figures 1, 2, and 3 show that, in the case of ungirdled cuttings with leaves, the hormone not only caused greatly increased rooting as compared with controls, but accelerated rooting considerably. There was no very significant difference in the total number of roots formed by apical and basal cuttings of terminal growth and by cuttings from the previous season's growth; apical cuttings of terminal growth, however, did form roots sooner than the other types of cuttings.

As shown in table I, the hormone not only caused increased production of roots on cuttings with leaves, but also caused root formation on cuttings without leaves, while on controls without leaves, no roots were formed.

[1] These investigations were conducted at the William G. Kerchoff Laboratories of Biology, California Institute of Technology, and the writer is indebted to the workers in these laboratories for supplying the hormone and other facilities.

TABLE I

ROOTING EXPERIMENTS WITH EUREKA LEMON CUTTINGS* WITH AND WITHOUT THE APPLICATION OF ROOT-FORMING HORMONES

LOT	TYPE OF CUTTING	LEAVES	HORMONE	GIRDLED	NO. OF CUTTINGS	AVERAGE NO. OF ROOTS PER CUTTING		
						AFTER 2 WEEKS	AFTER 3 WEEKS	AFTER 4 WEEKS
1	Tip of terminal growth	+	+	–	10	0.1	2.6	5.0
2	" " " "	+	–	–	5	0.0	0.6	1.6
3	Base of terminal growth	+	+	–	10	0.1	1.4	6.0
4	" " " "	+	–	–	5	0.0	0.0	1.1
5	Previous season's growth	+	+	–	5	0.0	2.0	4.2
6	" " "	+	–	–	5	0.0	0.0	0.8
7	Base of terminal growth	–†	+	–	10	0.0	1.1	1.6
8	" " " "	–	–	–	10	0.0	0.0	0.0
9	" " " "	+	+	+	10	0.0	0.0	0.4‡
10	" " " "	+	–	+	10	0.0	0.0	0.2§

* Set in sand in sash-covered propagating frame in greenhouse and shaded with burlap.

† Leaves removed at time of placing in sand.

‡ All roots occurred on two cuttings which had formed callus across girdle.

§ All roots occurred on one cutting which had formed callus across girdle.

FIG. 1. Rooting of hormone-treated apical cuttings from terminal growth of the lemon.

HALMA (1) reported the rooting of 4 lemon cuttings without leaves out of 27 tested, but stated that the amount of roots produced was insignificant. This, however, was not the case with hormone-treated leafless cuttings, because, as shown in figure 4, a really significant number of roots was obtained. This is considered as good evidence that the formation of roots is influenced by a supply of root-forming substance as well as by a supply of food material. Leafy control cuttings which rooted probably obtained their supply

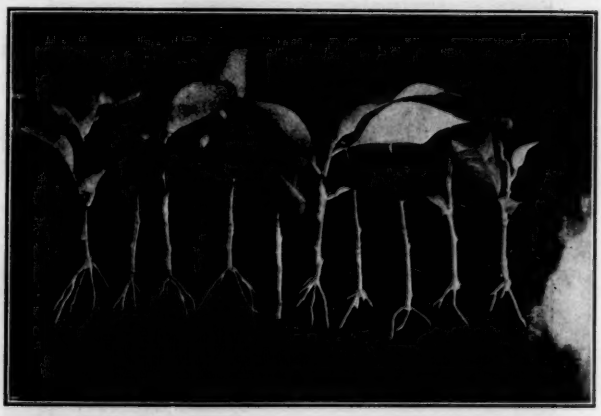

FIG. 2. Rooting of hormone-treated basal cuttings from terminal growth of the lemon.

FIG. 3. Rooting of control cuttings from apical and basal portions of terminal growth of the lemon: apical on right, basal on left.

of root-forming substance from the leaves where presumably it is manufactured.

It may also be noted from table I that girdled cuttings with hormone applied above the girdle did not form roots except in two instances where a callus had grown over the girdle and laid down new phloem tissue across the girdle. This evidence indicates that the hormone is transported in the phloem. Had the stem below the girdle been supplied with hormone, it

FIG. 4. Rooting of leafless lemon cuttings: right side, untreated; others, hormone treated.

would have rooted, as the results with leafless cuttings showed that stems without leaves will root when a supply of hormone is available.

These results with the rooting of lemon cuttings by hormone were so encouraging that experiments are now in progress with oranges and grapefruit, which are known to be more difficult to root than the lemon.

Experiments with cuttings of other plants

Results of experiments with cuttings of *Lantana*, fig, *Acalypha*, and *Tradescantia* are shown in table II. The *Lantana* cuttings were about 2 inches long and consisted of one internode and one node (at top) with two subtending leaves. They were placed in sand in an outdoor sash-covered frame on March 10, 1935. The hormone-treated cuttings produced a much larger number of roots than the untreated cuttings. Most of the roots on the untreated cuttings appeared at the base of the cutting, while on the treated cuttings roots were scattered all over the internode, appearing mostly at the point of application of the hormone, even when the hormone was applied to a portion of the cutting above ground.

Acalypha cuttings without leaves, planted in sand with bottom heat on March 19, 1935, had over six times as many roots on treated cuttings as on controls. As with *Lantana*, a large number of roots on the treated cuttings came out near or at the point of application of the hormone.

The results with the fig presented in table II were obtained from dormant cuttings placed in sand with bottom heat on February 19, 1935.

TABLE II

ROOTING EXPERIMENTS WITH CUTTINGS OF OTHER PLANTS WITH AND WITHOUT THE APPLICATION OF ROOT-FORMING HORMONES

PLANT	WEEKS AFTER PLACING IN SAND*	AVERAGE NUMBER OF ROOTS PER CUTTING	
		HORMONE-TREATED†	UNTREATED
Lantana	4.0	25.9	3.4
	5.0	35.7	3.8
	7.0	36.5	24.5
Acalypha	1.0	57.5	9.3
Fig	2.0	1.1	0.0
	3.0	8.9	5.7
	3.5	11.0	6.1
Tradescantia (internodes)	2.0	4.0	0.0
	2.5	6.3	0.0

* *Tradescantia* cuttings were placed in tap water, all others in sand.

† All treated and untreated lots consisted of 10 cuttings in all instances except *Acalypha*, which consisted of only 6 cuttings.

As with the lemon, *Lantana*, and *Acalypha*, the hormone-treated fig cuttings showed increased rooting as compared with controls.

Experiments with *Tradescantia* were made with internode cuttings without nodes and leaves. These, when placed in tap water and treated with hormone, formed roots within two weeks on the four internodes which were originally nearest the tips of the vine from which the cuttings were made, but did not form roots on the internodes which had been farther from the tip. No roots were formed on any of the untreated internodes.

These results show that treating with β-indolyl-acetic acid not only increased rooting with cuttings that will root when untreated, but also caused the formation of roots on leafless lemon and *Tradescantia* cuttings which do not ordinarily form roots. Other experiments are now in progress to determine the effect of this and other hormones on cuttings of other plants with particular attention to cuttings from plants which are difficult to root, such as the apple.

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THE STOMATA AND TRANSPIRATION OF OAKS

L. Edwin Yocum

(with two figures)

Introductory

Stomata and transpiration are of fundamental importance in the water relations of plants. It is evident that the loss of moisture in transpiration is equally as important as absorption of water by the root system in plant survival. Our mesophytic plants must have stomatal protection against excessive loss of moisture during drought. This is of paramount importance to us in our conservation work, where trees are being used as windbreaks, and to conserve moisture. Most of our knowledge regarding stomata has to do with herbaceous plants, as indicated by texts in plant physiology (Miller, **7**, and others). Growth habits and therefore soil conditions are not the same for leaf formation on herbaceous plants as on trees. On trees most of the leaves grow simultaneously in the spring while on herbaceous plants they appear over a longer period. In herbaceous plants stomata are commonly on both leaf surfaces, but tree leaves have them usually only on the lower surface.

Nearly all the water loss of plants occurs through the stomata. Some is lost through the epidermis, and since the upper epidermis has thicker cell walls, it is probable that a little more water loss occurs through the lower than through the upper epidermis. Water passes freely through the stomata when open; in fact Brown and Escombe (**2**) have shown that water may be lost through the stomata as rapidly as though it were evaporating from a free water surface instead of through many small pores. Sayre (**12**) has shown that the rate of transpiration depends more on the perimeter of the opening, than on its area, which is very important in the study of partly closed stomata. It is evident from their shape that the beginning of closure changes the perimeter very little, and only the last few per cent. of closure will in many cases affect the transpiration rate, as found by Jeffreys (**4**).

Methods

The relation of the stomata to transpiration was measured by the cobalt chloride paper method, since it seemed to offer a more accurate measure than the actual observational measurement of the final stages of closure in percentages. Mature leaves on one to three year old *Q. palustris, Q. rubra,* and *Q. prinus* seedlings grown outdoors, some in pots and some in seed beds, were used. Readings were taken at intervals of two hours or less over twenty-four hour periods.

Stomatal counts were made on fresh leaves of the above seedlings and from mature leaves from trees with the aid of the "ultropak" microscope at 500× magnification. REED (10) has shown that immature leaves of citrus have many more stomata per mm.^{2} than mature ones. At least ten leaves were used from two or more trees of each species. The trees were isolated individuals or at the edge of a group where they were freely exposed to light and wind. To select trees within a group would reduce the number of stomata, as SALISBURY (11) found the number to decrease with the protection of the forest.

The stomata were counted in a rectangle 200 μ by 150 μ, except in the case of the willow oak at 10 places on each leaf, or a total area of 0.3 mm.^{2} per leaf. Areas having veins thirty μ or more in width were avoided. In order to get the average number of stomata, five counts were made on each side of the midrib as follows: near the base of the leaf; near the midrib at the center of the leaf; near the center of the halfleaf; the edge opposite this point; and near the tip. Counts were made on willow oak leaves only at the base, middle, and tip.

Results

During the day, with adequate soil moisture the cobalt chloride paper reacted over the lower epidermis in one twentieth to one tenth the time required over the upper epidermis. At sunset the reaction time of the lower epidermis quickly changed to about one half the time on the upper side, but at sunrise it returned to daytime rates. Seedlings[1] grown in a seedbed in 1931 during a very dry period, when the soil moisture was below the wilting coefficient near the surface and a little above the wilting coefficient deeper in the soil, were found to have their stomata closed all day as indicated by their transpiration. The cobalt chloride paper required two hours or longer to change color on the lower surface and usually a little longer on the upper, due to the difference in epidermal cell walls. If they were entirely closed it was thought that carbon dioxide would be prevented from entering and that photosynthesis would be retarded. Leaves tested with iodine for starch at 4:00 P. M. on bright days gave a negative test for starch. A second seedbed was watered artificially and showed a high rate of transpiration and an abundance of starch in the leaves. Two days after a heavy rainfall the seedlings in the dry bed showed rapid transpiration and photosynthesis. A negative test for starch on a day favorable for photosynthesis appears to be a good test for all day total stomatal closure.

The differences in stomatal numbers for various parts of the same leaf were small, but the base of the leaves always had the fewest stomata; and in nearly all cases the region near the midrib had a lower number than the

[1] Results of studies made while employed by the Allegheny Forest Experiment Station, Philadelphia, Pa.

other parts of the leaf. No consistent differences were noted in the middle, edge, and tip counts, but in the total count the edge and tip were nearly 0.25 per cent. greater than the middle; the midrib, nearly 2, and the base, nearly 8 per cent. less. That is, in a general way the smallest number was found at the leaf base, and it increased toward the tip and edges of the leaf. Shaded leaves on mature trees and on seedlings always had fewer stomata than insolated leaves.

Stomata are small but very numerous and the guard cells may cover more than fifty per cent. of the surface as is shown by *Q. triloba* (fig. 1), and forty per cent. in the case of *Q. palustris* (fig. 2). Small areas of *Q. triloba*

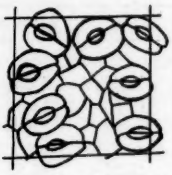

FIG. 1. *Q. triloba* Michx., stomata and epidermal cells in an area of 0.0036 mm.²

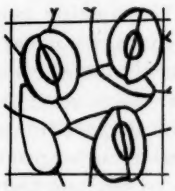

FIG. 2. *Q. palustris* DuRoi, stomata and epidermal cells in an area of 0.0036 mm.²

have an average of more than nineteen hundred per mm.², and the average for all counts was 1192 per mm.² This is exceeded to the writer's knowledge only by ADAMSON'S (1) report of *Veronica cookiana*, 2200. Other species of *Veronica* had an average number of 250 to 400 per mm.² Fully opened stomata of *Q. triloba* are about five μ long and one μ wide, or about

TABLE I

STOMATAL NUMBERS FOR *QUERCUS* SP. PER MM.²

TREES							
PLUSTRIS DUROI	*VELUTINA* LAM.	*RUBRA* L.	*STELLATA* WANG.	*PHELLOS* L.	*PRINUS* L.	*COCCINEA* WANG.	*TRILOBA* MICHX.
PIN	BLACK	RED	POST	WILLOW	CHESTNUT	SCARLET	SPANISH
497	580	680	693	723	890	1038	1192
SEEDLINGS							
539		836	635				

one per cent. of the total surface of the leaf. Table I gives the average stomatal numbers for trees of eight species, and three seedlings.

Stomatal counts were made at different elevations on two species of oaks, *Q. palustris* at 7, 17, and 60 feet, and *Q. velutina* at 7, 14, and 30 feet, taken from all sides of exposed trees. The results were similar for the two species. The data given for *Q. palustris* in table II are the average of 100 or more

TABLE II

STOMATAL NUMBERS FOR A *QUERCUS PALUSTRIS* DUROI TREE AT THREE ELEVATIONS

HEIGHT ON TREE	AVERAGE NUMBER PER 0.03 MM.² FOR EACH REGION OF LEAF						
	MIDDLE	EDGE	TIP	MIDRIB	BASE	TOTALS	PER MM.²
ft.							
7	15.85	15.85	15.57	15.36	14.21	2154	511
17	14.50	14.20	14.70	14.65	13.65	1434	481
60	15.40	14.85	15.30	14.60	14.00	1482	494
Average	15.31	15.08	15.25	14.88	13.96		496

counts for each elevation. The number per mm.² for each elevation is within three per cent. of the average number and while at 7 feet high the averages are higher, it is probably not significant.

In order to examine further the relation of elevation to stomatal number the shoot of a three year old *Q. palustris* seedling was removed at the ground level in the winter, and a single sprout allowed to grow the following spring. On July 19, the sprout was forty-four inches tall. Stomatal counts were made at eight inch intervals from the ground as follows:

Height from the ground, inches	8	16	24	32	40
Stomata per mm.²	410	430	450	503	533

The leaves at the forty inch level were mature and had developed during July. This shoot had developed its leaves progressively as is common for herbaceous plants.

Discussion

The transpiration rate of the oaks studied showed that the stomata normally remain open throughout the day but close at sunset. With low soil moisture the stomata may remain closed all day even to the extent of preventing photosynthesis. This action of the stomata is very important as it indicates a maximum transpiration rate when soil water is available, justifying the statement sometimes made that "trees are our natural humidifiers"; and when soil water is so limited that many herbaceous plants would be seriously injured or killed, trees may resist drought for some time

because of the action of their stomata. Thus in addition to improving the physical conditions of the soil for the retention of soil moisture the oaks give off water rapidly when it is abundant in the soil but withhold it during drought. MAGNESS (5) has reported similar drought reactions for apple trees in the eastern states, and finds that there is a cessation of growth of fruit, but that the stomata rarely remain open later than noon on bright clear days, regardless of the soil moisture conditions.

This difference in afternoon stomatal condition suggests interesting questions regarding the rate of photosynthesis in apples and oaks. Is the rate much higher for apples in the morning than for oaks, or is the total amount greater for oaks? Of equal interest and perhaps more important to conservationists is the "water requirement" of trees, or as MAXIMOV (6) prefers to state it, the "efficiency of transpiration." Are we more interested in the maximum humidifying power of the plant or in the maximum plant material produced with the available water? SCARTH (13) has discussed the problem of the stomatal influence on transpiration and photosynthesis.

The stomatal counts for the species recorded were made during the summer of 1934. During the previous six months, December to May, inclusive, the total rainfall was greater than normal by more than six inches; during only two months, January and April, was it slightly below normal, according to the U. S. Weather Bureau records for Washington. Excessive rainfall during the spring tends to decrease the stomatal numbers, according to HIRANO (3); therefore the frequencies given in table I are lower than the number to be found during a dryer spring. The number of stomata found on trees growing in a dense forest would probably be lower because of the high percentage of shaded leaves, which were found to have fewer stomata. HIRANO (3) points out some ecological relationships to stomatal numbers in citrus. The oak species examined in this study have not indicated a well defined ecological relationship; however, the three species having the greatest frequency grow on dry soil.

Trees generally have a higher stomatal frequency than shrubs or herbaceous plants as found by SALISBURY (11), RABER (9), and others. Citrus has been studied extensively by HIRANO (3), OPPENHEIM (8), and REED (10), who found a frequency often more than 500 per mm.^{2} and a distribution similar to that found in oaks. No record has been noted of a group of species with as high stomatal frequencies as are found here in the oaks.

SALISBURY (11), YAPP (14), and others have found a rapid increase in the stomatal numbers as the leaves are higher on the stem. REED (10) finds a certain relationship on long stems of citrus but not on short ones. MAXIMOV (6) describes YAPP'S work under the heading "Leaf structure in relation to water," but follows the suggestion of ZALENSKI that the later leaves

have more water deflected to the lower leaves, causing a greater osmotic pressure in the upper leaves, producing more stomata. It has been shown by HIRANO (**3**) that the amount of spring rainfall has more influence on stomatal number than has annual rainfall. Herbaceous plants grow their leaves during several months. In YAPP'S (**14**) study of *Spiraea*, illustrations are given from March to June, inclusive, during which time the external conditions favor the increase of transpiration as new leaves develop, but normally the soil moisture becomes less. This would cause the osmotic pressure of the leaf cells to become greater and the leaves to become more xeromorphic, with increased stomata. The difference in height would have relatively no effect compared with the difference in the resistance which the soil offers to water loss in early spring and in the dryer summer period. This would seem to explain the fact that stomatal frequency varies so little on the *Q. palustris* tree with a height of sixty feet since the leaves all developed with nearly the same soil moisture; but a seedling of the same species has thirty per cent. more stomata at forty inches than it has thirty-two inches lower on the same stem.

Summary

1. The stomata of oaks are functional and may remain completely closed during a drought.

2. Stomatal frequencies on eight species of oaks are all high, and on *Q. triloba*, the highest, the frequency is exceptionally high.

3. Stomatal numbers do not appear to increase with height on mature, exposed trees, but they do appear to increase on indefinitely growing stems.

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PHOTOSYNTHESIS IN *GRIMMIA MONTANA*[1]

ELIZABETH MCKAY

(WITH ONE FIGURE)

Introduction

The xerophytic moss, *Grimmia montana,* is known commonly as "black moss." During the greater part of the year the name is fully justified, for when dry the moss appears on the dark basaltic rock outcrops of southern Washington as a small black cushion 1–6 cm. wide, composed of a dense tuft of plants from 1 to 2 cm. high. The tuft usually shows slightly gray, owing to the presence of long hyaline points on the leaves. In addition to the long transparent points, *G. montana* shows a number of other xeromorphic characters (**13**). The margins of the leaves are revolute and the leaves are slender and pointed, with small, thick walled cells. The plants grow in dense cushions, reducing the surface exposed to insolation.

When the moss is dampened the changes produced are immediate and marked. The leaves unfold, the plant appears to expand, and the dull gray-black color is replaced by a vivid green. Apparently this change in appearance is followed shortly by a decided activation of life processes in the plant.

The object of this investigation was to attempt to determine the time elapsing after the addition of moisture to the air-dry plant before photosynthetic activity begins.

Literature

FRAYMOUTH (**3**) quotes BASTIT (1891) and JONSSON (1894) as having found that dry mosses respire and that respiration increases with increase of the water content.

IRMSCHER (**6**) concludes that the drought resistance and the marked changes of a given species of moss in different habitats are due to the great adaptability of the protoplasts to changes in environment. This characteristic is not great enough for the survival of rapid alternate desiccation and moistening. In addition to the peculiarity of the protoplasts, the mosses possess dormant buds which survive more severe conditions than the ordinary cells and develop new protonema when conditions are favorable.

DAVY DE VIRVILLE (**1, 2**) has grown several species of moss and has noted the effects of light, temperature, and humidity on the structure of

[1] Contribution no. 40 from the Botany Department of the State College of Washington.

the plants. His conclusion is that, even though a moss is normally xerophytic, high humidity is more favorable to its growth.

MAYER and PLANTEFOL (**9, 10, 12**) find that mosses retain some water even at 100° C. In *Hypnum triquetrum* this amounts to 5–6 per cent. They find also that a moss with water content of 8 per cent. will absorb water to 18 per cent. of its weight when placed in a saturated solution of NaCl. From these data they conclude that the force involved in resisting desiccation is imbibitional rather than osmotic. Photosynthetic activity of *H. triquetrum* increases with increase of water content up to 300 per cent. Respiration increases up to 150 per cent. Decrease in the water content causes a more rapid decrease in photosynthesis than in respiration, so that apparently respiration is less directly dependent upon the water content than is photosynthesis.

By estimation of the oxygen absorbed, FRAYMOUTH (**3**) has correlated the increase of respiration with increase of water in several lower plant forms, including algae, lichens, and the moss *Hypnum cupressiforme*. She finds that respiration in *H. cupressiforme* increases with increase of water content up to 300 per cent. of water.

ILJIN (**5**) has studied numerous vegetative cells, both resistant and non-resistant to desiccation, and concludes that the hardening of a plant is a process of devacuolization. The ability of a cell to withstand desiccation is due to lack of vacuoles, and the reason the Grimmiaceae are resistant is that they lack these structures.

Material and methods

FIRST METHOD.—Two methods were used in determining the beginning of photosynthetic activity in *Grimmia montana*. In the first method the moss was immersed in water and the change of resistance of the water was measured with the submerged moss exposed to bright sunlight. Redistilled water was employed, but the purity of this water did not approach that necessary for exact quantitative tests, as is shown by variation in the measurements from 10,125 to 12,375 ohms. The water was kept in the laboratory from a few hours to several days. During this time it was stored in glass flasks with cotton stoppers so that the atmospheric carbon dioxide could dissolve freely. A second measurement was made duplicating the conditions except that the moss was placed in darkness. It was believed that the plant placed in darkness would show, owing to respiration, a gradual increase in the carbon dioxide evolved and hence in the conductivity of the solution in which it was submerged. The plant which was exposed to sunlight, and was therefore free to carry on photosynthesis, would utilize some of the carbon dioxide produced and show either a smaller decrease of resistance than that shown by material kept in darkness

or an actual increase of the resistance. The length of time elapsing before the darkened and illuminated samples showed a difference of resistance was taken as a measure of the time necessary for the beginning of photosynthesis. In order to check the possibility of resistance changes due to solution of carbon dioxide from the atmosphere of the greenhouse, tests were run on the distilled water without moss, through similar periods. These samples showed no measurable changes of resistance.

Material for the resistance tests was collected during the summer season. At that time the moss had received very little moisture for several weeks and was practically air-dry. As nearly as possible the material was collected from places exposed to direct sunlight during either the morning or afternoon, and those tufts were chosen which appeared to have the same degree of hyaline leaf point development. In the laboratory the tops were cut from the plants, leaving the rhizoids and as much as possible of the earth in which they were imbedded as waste material. The material that remained, after trimming, included the tops of the stems with the living leaves.

The leafy stems were then washed and rinsed several times with distilled water in order to remove all possible material which might contain electrolytes and cause resistance changes. After being washed, the tops were air-dried and weighed in samples of 1 gm. each. Before weighing, any remaining foreign material was carefully removed from the sample. Between the time of preparation and the time at which the moss was used, it was stored in glass bottles. In determining the water content, samples were dried, in accordance with the recommendations of LINK and TOTTINGHAM (**8**), at 65° C. to a constant weight. The loss was found to be 0.2 gm. from a sample weighing 4 gm., or 5 per cent. of the air-dry weight after 42 hours' drying.

The sample, after being washed and dried, was placed in a measured quantity of distilled water in a 250-cc. beaker and kept at a temperature of $24° \pm 0.5°$. Using a wheatstone bridge, measurements were made of the resistance changes in the water at short intervals for about two hours following immersion of the material. The moss was stirred frequently, to expose all parts to the sunlight and to distribute the CO_2 uniformly throughout the medium. In the same manner, samples were placed in water and the change of resistance was measured, except that the beaker was surrounded by several thicknesses of black cloth, to exclude the possibility of photosynthesis. In this case also the solution was stirred frequently and the temperature maintained at $24°$ C. $\pm 0.5°$. The results obtained by these resistance measurements are only qualitative, because of several uncontrolled factors. The intensity of the sunlight varied with the different determinations, and, owing to the occasional passage of a

cloud, during each determination. The original purity of the water varied somewhat and the nature of the impurities was not ascertained. This offers opportunity for change of resistance resulting from reactions of the carbon dioxide, such as the formation of ammonium carbonate, rather than directly from the carbon dioxide changes. A third possibility of error lies in the leaching of electrolytes and buffer solutions from the cells of the plant, and the dissolving of material not removed from the exterior of the plant by washing. The solution of carbon dioxide from the atmosphere was shown by resistance measurements of the distilled water to be insufficient to produce any measurable change in resistance.

SECOND METHOD.—In this method measurement of the increase in reducing sugars was used as an indicator. The moss was collected during the period when moisture was relatively abundant and the plant was growing. The moss was placed in darkness, while damp, and allowed to dry slowly to air-dryness. Preliminary comparison of the sugar content of samples dried more rapidly in light with those dried in darkness showed that, in the latter, the excess sugar was to a large extent used by the plant while photosynthesis was not being carried on. The samples were allowed to remain in the air-dry state for several days before tests were made. A small piece of the moss cushion was then selected and a microchemical sugar test was made on the dry material, by placing a few leaves on a glass slide, adding a few drops of Fehling's solution of HAWK'S formula (**4**), and warming. The leaves were observed under a microscope and the amount of precipitate formed at definite time intervals was noted.

After a few dry leaves had been tested, the piece of moss was dampened well and placed in bright sunlight, in a watchglass containing a small amount of tap water. Sugar tests were made and the relative amounts of precipitate were recorded. It was found that leaves of the stem tip bearing archegonia contained more sugar than those of a vegetative stem tip under the same conditions, so care was used in the tests to obtain leaves of the same kind for all tests of a single sample.

Results and conclusions

The results obtained by the measurement of the change of resistance are shown in figure 1, which is a representative curve chosen from a number of determinations. Measurements were made using 1-gm. samples of dry moss in 225 cc. of water at a temperature of 24° C. The solid line shows the change of resistance of the solution surrounding a moss sample when the moss is exposed to sunlight; the dotted line shows the change when the moss is kept in darkness.

The first 10 minutes show an abrupt decrease in resistance, apparently due to the solution of material not removed from the moss in washing or

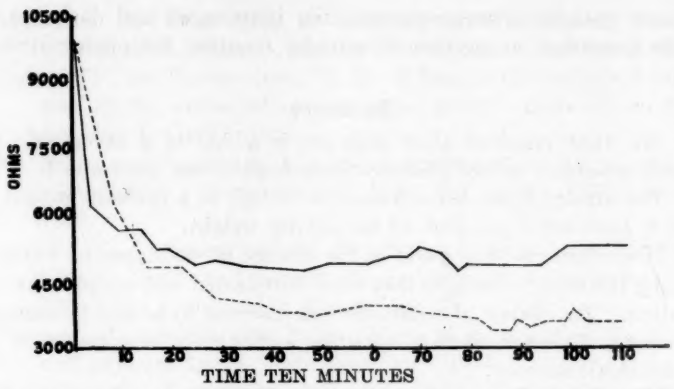

FIG. 1. Changes in resistance of the medium during photosynthesis and respiration of *Grimmia montana*.

to the leaching of cell contents. In the period from 10 to 20 minutes the curves flatten and differences in resistance begin to show between the two. The curves show minor fluctuations, but the darkened sample shows a gradual lowering, expressing the increase of carbon dioxide from respiration in the absence of photosynthesis. The curve for the illuminated sample reaches a level between 30 and 40 minutes, showing that the photosynthetic reaction has apparently reached an equilibrium with the respiratory process and atmospheric absorption, resulting in a nearly constant carbon dioxide supply.

The conclusion is reached that photosynthesis begins in *Grimmia montana* from 10 to 20 minutes after the plant is immersed in water and illuminated, and that within 25 minutes the activity is great enough to utilize the carbon dioxide produced in respiration as well as that supplied by the atmosphere. Within 40 minutes the plant apparently reaches a photosynthetic equilibrium for the given temperature and light conditions.

The quantities of reducing sugars in the moss after receiving moisture and light show no quantitative change in the cells of the leaf within 5 minutes. Within 7 or 8 minutes a slight difference is shown. After 10 minutes the increase is decided, and after 25 minutes, fairly large. At the end of 30 minutes there is no further increase, indicating that the process has reached an equilibrium, possibly because of the accumulation of photosynthetic products.

The microchemical tests show a somewhat shorter period before photosynthesis begins than is shown by the resistance measurements. This is probably explained by the slight buffer action of the carbonic acid, which would retard the change of resistance so that the time found in the comparison of

resistance changes in water surrounding illuminated and darkened moss may be somewhat longer than is actually required for photosynthesis to begin.

Summary

1. The time required after moisture is added to a xerophytic moss, *Grimmia montana,* before photosynthesis begins, was determined.

2. The air-dry moss, when heated at 65° C. to a constant weight, was found to have lost 5 per cent. of the air-dry weight.

3. Measurements were made of the change of resistance of water surrounding the moss in samples that were illuminated and samples that were kept dark. The change of resistance was assumed to be due to changes in the amount of carbon dioxide, resulting either from respiratory or from photosynthetic activity.

4. Microchemical tests were made using Fehling's solution to determine the change in amount of reducing sugars in the moss after being dampened, as a measure of the time required for photosynthesis to begin.

5. Photosynthesis apparently begins 6 to 10 minutes after water is supplied to the moss, is great enough within 25 minutes to utilize the carbon dioxide produced by respiration, and reaches an equilibrium within 30 to 40 minutes.

The writer wishes to thank Dr. F. L. Pickett for suggestion of the problem and helpful criticism of the work.

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SOME CHEMICAL ASPECTS OF CALCIUM DEFICIENCY EFFECTS ON *PISUM SATIVUM*

DOROTHY DAY

The beneficial effect of calcium in the growing of crops has been known through the ages but it is only in modern times that any detailed investigations have been made to see just how this element affects the individual plant or its parts. Various criteria have been used, usually based on the external appearance of the plant, but more recently attention has been turned to the effect on the internal anatomy caused by lack of this element, as in the work of SOROKIN and SOMMER on the pea plant (**5**). WARINGTON (**6**) has approached the problem from the chemical point of view with quantitative estimations of the amount of calcium absorbed by *Vicia faba* at different stages of growth and under varying nutritional conditions.

It has been the object throughout this research to combine and correlate the anatomical aspect with the chemical. This was begun by growing all of the plants two in a pot so that both received exactly the same treatment. Thus it was possible to examine the internal structure of one plant and to use the other for determinations of weight and of calcium content. A preliminary report summarized all results (**1**). Surprizingly enough, an anatomical examination (**2**) of plants treated with various solutions showed almost no variation in the internal structure of root and stem even when calcium was completely withheld, and the chief difference between plants so unlike in size and succulence was in the amount of elongation of their several organs.

Methods

Canada field peas (*Pisum sativum* L.) were grown in sand cultures in the greenhouse under controlled conditions. Details of the methods and materials have been given previously (**2**). Seven kinds of nutrient solutions were used to meet the mineral requirements of the plants, six pots to a treatment. These solutions can be grouped in three categories according to their composition and also according to their physiological effect: A, those with an optimum of calcium; B, D, F, those with one-half that amount; and C, E, G, those with no calcium. Solution A is the one that preliminary experiments had shown was best suited to the growth of this species (**2**). In solutions A, B, and C the only difference is in the amount of calcium nitrate; solution B contains one-half the quantity present in A, while C lacks it entirely. In solutions D and E this same ratio holds but the nitrate content is compensated in chemically equivalent amounts by equal parts of sodium nitrate and potassium nitrate. In solutions F and G

there is a similar substitution for the nitrate, but one-fourth of the nitrate is replaced by magnesium nitrate and three-fourths by potassium nitrate; therefore there is a substitution for the nitrate radical rather than a molecular substitution.

All plants were harvested at the end of a five weeks' period of growth, just before the death of the plants grown without calcium, although those given one-half the full amount of this element were still fairly vigorous and probably could have lived for some time longer. From each pot one plant was fixed for anatomical observations and the other was used for fresh weight and dry weight determinations of shoots and of roots. After drying, this material was pulverized preliminary to the quantitative analysis for calcium, which was performed with duplicate samples according to McCRUDDEN's method (**4**). Results were recorded in terms of calcium oxide.

Results

In general, the plants of the complete nutrient solution (A) seemed to be fairly uniform in height with an average of twenty-eight inches above the cotyledonary node. Those plants given the solutions with one-half the amount of calcium (B, D, F) form a fairly uniform group and were almost as tall as those with the complete nutrient solution (A). There was, however, a decided difference between any of these and the plants deprived of calcium (C, E, G), which formed a third distinct group; these were about one-half as tall as those given the complete nutrient solution (A).

The shoots of those plants receiving the full ration of calcium (A) showed the greatest average fresh weight, while those given one-half the amount of calcium (B, D, F) were almost as heavy. However, those starved of calcium (C, E, G) were less than one-third as heavy as the plants treated with the solution containing the one-half ration of calcium. Roots of these same plants showed similar relationships except that there was not quite so decided a difference between the plants starved of calcium and those given the half amount of this element although it was still marked. Details of these weights are given in table I.

The greatest average dry weight of shoots was shown by the plants receiving the complete nutrient solution (A). Those given some calcium (B, D, F) were slightly lighter, while those deprived of this element (C, E, G) were definitely the lightest, although the differences were not so marked in any case as those noted for the fresh weights. Roots of these same plants showed similar results except that the difference is even less between the plants starved of calcium and those given some. The data are included in table I.

The percentage of dry matter in the shoots is greatest in the plants starved of calcium and least in the plants given the most calcium. The

TABLE I

FRESH WEIGHT, DRY WEIGHT, AND CALCIUM CONTENT OF CANADA FIELD PEAS (*PISUM SATIVUM L.*)

NUTRIENT SOLUTIONS	COMPLETE	ONE-HALF CALCIUM			NO CALCIUM		
	A	B	D	F	C	E	G
		$Ca(NO_3)_2$ UNREPLACED	$Ca(NO_3)_2$ REPLACED BY EQUAL PARTS OF $NaNO_3$ AND KNO_3	$Ca(NO_3)_2$ REPLACED BY NITRATE MIXTURE $\frac{1}{4}$ $Mg(NO_3)_2$ AND $\frac{3}{4}$ KNO_3	$Ca(NO_3)_2$ UNREPLACED	$Ca(NO_3)_2$ REPLACED BY EQUAL PARTS OF $NaNO_3$ AND KNO_3	$Ca(NO_3)_2$ REPLACED BY NITRATE MIXTURE $\frac{1}{4}$ $Mg(NO_3)_2$ AND $\frac{3}{4}$ KNO_3
SHOOTS							
Av. fresh wt. gm.	12.163	10.090	11.003	10.684	3.371	3.276	3.104
Av. dry wt., gm.	1.339	1.213	1.295	1.234	0.506	0.480	0.425
Per cent. of dry matter	11.009	12.022	11.770	11.550	15.010	14.652	13.692
Av. CaO per plant, gm.	0.028	0.023	0.019	0.023	0.010	0.009	0.009
CaO, per cent. of dry wt.	2.125	1.900	1.500	1.825	2.000	1.850	2.200
CaO, per cent. of fresh wt.	0.230	0.228	0.173	0.206	0.297	0.275	0.290
ROOTS							
Av. fresh wt. gm.	6.629	4.735	5.933	5.601	2.093	2.611	2.371
Av. dry wt., gm.	0.591	0.372	0.515	0.496	0.165	0.204	0.170
Per cent. of dry matter	8.915	7.856	8.680	8.856	7.883	7.813	7.170
Av. CaO per plant, gm.	0.013	0.009	0.013	0.008	0.004	0.004	0.006
CaO, per cent. of dry wt.	2.250	2.500	2.500	1.550	2.665	1.940	3.330
CaO, per cent. of fresh wt.	0.196	0.190	0.219	0.143	0.191	0.153	0.253

contrary is true for the roots but the difference is slight. The exact proportion of dry weight to fresh weight in each case is shown in table I.

The plants given the most calcium in the nutrient solution contained the most at the end of the growth period of five weeks. For both shoots and roots the plants treated with the half ration showed three-fourths as much calcium as those plants offered the complete nutrient solution. Those deprived of this element had almost one-third as much calcium as those with the complete nutrient solution. In general, the plants having equal amounts of calcium in the various nutrient solutions were similar. However, decidedly less calcium was present in the shoots of solution D (one-half calcium) and in the shoots and roots of solution E (no calcium). These were the solutions in which the calcium nitrate of the complete nutrient solution was replaced by equal parts of sodium nitrate and potassium nitrate.

Calcium oxide, computed as percentage of the dry weight of the plant, is generally greater in the roots than it is in the shoots. It is greatest for the roots of the plants deprived of calcium and least for the shoots of plants given the one-half amount of calcium. It is lower in shoots from solution D and in plants from solution E as has been noted for the entire plant.

Calcium oxide, calculated as percentage of the fresh weight of the plant, is higher for the shoots, in general, than it is for the roots. This is the reverse of the relation just noted in which the roots show more calcium as percentage of dry weight. The percentage of fresh weight is greatest for the shoots of plants starved of calcium while the roots of these plants show almost no difference from the plants given the complete nutrient solution.

Discussion

An optimum of calcium increases both the number of internodes and the length of the internodes, and increases the fresh and dry weight in comparison with plants given only one-half as much calcium. In all these respects the difference is even more striking between fully nourished plants and those deprived of calcium.

Calculations of the dry weight in relation to the fresh weight show that, for the quantities of calcium used here, the roots have a slightly greater proportion of dry weight according to the amount of calcium present in the nutrient solution. Probably there is some storage of material without much leaching in the presence of calcium. For the shoots the greater the amount of calcium in the nutrient solution the less is the percentage of dry matter and the greater is that of water present. This appears to indicate that calcium is conducive to succulence. However, other workers have ascribed this same result to other elements, especially nitrogen, used in similar experiments. With the possible exception of nitrogen, might it be said that

succulence should be associated with good nutrition in general rather than with the presence of any particular substance? This seems to be the contention of FAMIN (3), who disagreed with VESQUE, VAN RYSSELBERGHE, and PETRIE since he found that the majority of salts which he studied do not increase the succulence of young and vigorous tissues.

Since most calcium was present in the complete nutrient solution it is not surprising that the roots and stems grown there should show the most calcium oxide per plant. The close approach made to this by the plants given only one-half that amount of calcium may indicate that a plant uses most of the material stored in the seed, that it makes the most of any calcium supplied, that it needs little calcium, or all three. The relatively large amounts of calcium oxide present in the plants grown in the solutions deficient in calcium may support one or more of these points. It must be remembered that all plants were harvested just before the death of these last groups of plants although the partially starved plants were still fairly vigorous and would have lived for some time longer. WARINGTON (6), working with *Vicia faba*, also found that the amount of calcium absorbed is proportional to the calcium supplied in the nutrient solution.

Summary

1. Canada field peas were grown under greenhouse conditions in nutrient solutions in which the proportion of calcium nitrate was varied. External appearance, fresh weight, and dry weight were observed and measured. Samples were analyzed for calcium content according to MCCRUDDEN'S method.

2. These results support the contention that lack of calcium affects the external appearance of pea plants, making them shorter and less succulent.

3. Both shoots and roots of the plants given the complete nutrient solution showed the greatest fresh and dry weights; those given a half ration of calcium were almost as heavy, and those deprived of calcium were decidedly lighter.

4. The percentage of dry matter in the shoots is greatest in the plants deprived of calcium. The contrary holds true for the roots but the difference is slight.

5. The plants given the most calcium in the nutrient solution contained the most at the end of the growth period of five weeks. Those starved of calcium had almost one-third as much calcium as those grown in the complete nutrient solution.

6. There was less calcium in the plants in which the calcium nitrate of the complete nutrient solution was replaced by equal parts of sodium nitrate and potassium nitrate than in those grown in solutions in which the calcium nitrate was not compensated or was replaced by magnesium nitrate and potassium nitrate.

7. There is no consistent variation in the proportion of stored calcium to fresh weight or to dry weight.

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SOME EFFECTS OF FUEL OIL ON PLANTS

GEORGE D. FULLER AND MARGARET R. LEADBEATER

Some years ago a storage tank of fuel oil emptied upon the soil is supposed to have caused the death of several oak trees in a suburb of Chicago. This caused the writers to make certain preliminary experiments on the action of such oil on certain potted plants.

While attention had been directed towards the entrance of such oils into the leaves of plants when applied as a spray (see among others GINSBERG (2), KNIGHT *et al.* (4), KELLEY (3), DE ONG (1), and ROHRBAUGH (5)), no investigation regarding their effects upon roots is known to the writers.

The fuel oil used was purchased from the Consumers Petroleum Company, Chicago, Illinois. It is described commercially as 3236 Baume gravity gas oil, consisting principally of saturated hydrocarbons. It is obtained in the refining of petroleum as one of the heavier fractions of distillation. The viscosity is about 55 seconds Saybolt. In appearance it is medium thin, of a greenish amber color, and is similar to the "medium oils" which spray investigators have found most useful.

The experiments were carried on with four plants: the tomato, *Lycopersicum esculentum;* the peach, *Prunus persica;* the apple, *Pyrus malus;* and *Ageratum houstonianum.* The four were chosen for their availability rather than for any scientific reason, but they proved suitable material for the investigation because none of them contained natural oil in sufficient quantities to interfere with the recognition of the introduced oil in the plant body, and they gave an interesting variety of reactions. As the most extensive experiments were with tomatoes and peaches, the reactions of these two species only are being reported.

The tomatoes were from greenhouse stock, all from the same planting, carbohydrate high and tending to reproduction. The peaches and apples were year-old seedlings obtained through the courtesy of Dr. M. J. DORSEY, Chief in Pomology at the University of Illinois. They were planted in good potting loam, the tomatoes being in 5-inch pots and the peaches in 6-inch. Two lots of 12 plants each were used for each species divided into sets of two pots each. One of these sets, without oil, served as the control; the other five sets had one application of oil applied to the surface of the soil in amounts of 1, 2, 3, 4, and 5 per cent. by volume of the soil content of the respective pots. In one lot of each species the soil was heaped about the stems of the plant so that there was no surface contact of the oil with the stems. This was designated as the *M* series; the others constituted the *S* series. All plants were watered regularly to maintain proper moisture conditions for good growth and the plants were kept under observation for 51 days.

Results with tomatoes

The control plants and part of those in soil with 1 per cent. of oil appeared quite healthy and vigorous at the end of the period. The plants of the *M* series usually showed injury a day or two before those of the *S* series.

The first signs of injury were the wilting of the youngest two or three leaves of the main axis and those of the lateral axes. The whole tip sometimes wilted with the young leaves. Leaf wilting occurred with or without discoloration. In case of discoloration, the leaves turned either olive-green or yellow. No oil was found in leaves which had turned olive although it looked from the outside as if it might be present. Flowers developed if the buds were fairly mature when injury became apparent, otherwise they blighted.

Yellowing of the older leaves was not taken as a symptom because the plants stood rather close together and the checks dropped their oldest leaves also. However, as the injury advanced all the leaves of the plants yellowed, wilted, and died. Sometimes the stem collapsed near the surface of the ground or halfway up the stem, sometimes it remained upright. This seemed to be a matter of individual resistance for there was no correlation between doses of oil and methods of degeneration. Wilting occurred in from eight to fourteen days for the larger doses and twelve to twenty for the smaller ones. There was less difference in the time of death of the two groups. It occurred in twenty-two to twenty-eight days in the plants in soil with 4 to 5 per cent. of oil, in twenty-four to forty-two days for those with 3 per cent. or less, and half the plants in soil with 2 per cent. of oil were still alive at the end of the experiment.

Sections were made with the use of the freezing microtome from plants of both the *M* and *S* series grown in soil with 1, 3, and 5 per cent. of oil. These sections were taken from the tips of the plants, from the mid-stems, from the stems at the surface of the soil, and from the roots. They were stained with Sudan III and examined for the presence of oil, which was found in varying amounts in all the plants, with larger amounts in plants from soil with highest oil content.

The oil was found most regularly in the xylem, particularly the primary, where the stain brought out small drops clinging to the sides of the vessels or completely clogging them. Only where it was plentiful did it spread to the xylem parenchyma or to the rays, being confined to the bundles except when present in excess. It was next most prominent in the intercellular spaces of the pith and cortex. In the root, it was about equally distributed between xylem and cortex, but it was often difficult to tell about the location because degeneration had proceeded so far that the structures were but ill defined. Judging by color and texture, the roots were certainly badly injured by the oil, although they did not always show great quantities of it within them. Roots of check or low-quantity treatment plants were cream

to light tan in color, firm, and brittle. Those severely affected by higher percentages were dark red-brown to black, withered, and limp.

Results with peaches

Almost a month elapsed before the peaches showed any response to the oil treatment. A very small plant (*M* 5 per cent.) was wilted when visited on the twenty-fifth day. Very soon afterwards all but one of those at 2 per cent. and above followed suit. There was no discoloration. The leaves wilted and died, green. There was no outstanding difference in time between the 2 and the 5 per cent. plants nor between *M* and *S* plants. Five of the eight *M* trees showed injury at thirty-seven days, four of these dying after forty days. There were also five *S* trees injured thirty-seven days after treatment, taking forty-three to forty-five days to die. All plants in soil with 2 per cent. of oil or more were dead in forty-five days.

When sections of all representatives of the series were examined, they were uniform in giving a positive stain for oil only in the cuticle. This fact remained a puzzle until the root systems were exposed in discarding the plants. One per cent. of oil seemed below the critical amount, and the plants which grew in soil with this dosage were alive at the end of the experimental period, and had almost as good root development as the check. But somewhere between 1 and 2 per cent. the lethal concentration is reached. From 2 per cent. upward the tops were somewhat stunted, and the roots had practically disappeared. Only a few limp, brown, straggling ends of the branches were visible. The control plants showed that their roots had elongated and had branched freely during the time of the experiment. In contrast with these the plants in soil with 2 per cent. or more of oil showed no root growth, but a depletion and decay of the original root system. The direct damage by the oil seemed, in the peaches, to be confined entirely to the underground parts but with equally fatal effects.

Discussion

The species examined exhibited marked variation in their reaction to the oil treatment. Where oil actually entered the tissues a slow death followed. This was probably a toxic action since no sections seemed to show oil in enough cells seriously to impede ordinary translocation. Where oil had apparently not gained entrance to the plant, as in the peaches, some other factor must have been responsible. Either there was local toxic action at the roots, or the spread of oil about them was sufficiently complete to prevent translocation of water in the soil and its absorption by the roots, and the plant literally died of drought.

It is not known what relation the oil bears to the soil. A small quantity is evidently held among the soil particles by capillarity, since it is only where larger amounts are added that the oil seemed to move through the soil. It has been shown that as roots take in water from soil adjacent to

them, free water will move in from more remote areas to replenish the supply. This would continue if necessary until only the hygroscopic water remained. Where 3 per cent. or more of oil was added to a pot it was found concentrated about the roots, which would indicate that a similar situation obtained with it.

If some definite part is held by the soil particles it would explain the failure of the lowest quantity to produce results. In the case of tomatoes and peaches the soil seemed able to take up about 1 per cent. of oil by volume without injuring them; but when the amount was greater, the effect was detrimental to the plants.

This investigation opens up many questions particularly of physiological interest which must be left for future studies. Probably abnormalities in temperature, humidity, water content, and nutritive relations would have special meaning when plants are subjected to a test of this sort. It will be valuable to correlate results of outside experiments with the greenhouse data.

Summary

1. Commercial fuel oil has a harmful effect on plants when brought into contact with their roots, through the soil.

2. The effect becomes fatal when the quantity is raised above the critical point for a species.

3. The effect may or may not be due to penetration.

4. If the oil has penetrated the tissues, it is most commonly found in the primary xylem; to a lesser extent it enters the secondary xylem, and the intercellular spaces of the pith and cortex.

5. Contact does not seem to be a primary cause of death.

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BRIEF PAPERS

EFFECT OF TITANOUS CHLORIDE ON THE FORMATION OF CHLOROPHYLL IN *ZEA MAYS*[1]

(WITH ONE FIGURE)

Titanium is found as a constituent of many ores, the chief one being ilmenite and rutile. As the oxide it is generally distributed in surface soils. Clay soils often contain from 0.3 to 0.6 per cent. TiO_2. Titanium stands in tenth place in the series of elements which make up the earth's crust. Many plant organs have been found to contain TiO_2. Potatoes may sometimes contain as much as 0.336 per cent. of their dry weight.

NEMEC and KAS (**2**) in 1923 found that increased crop yields could be obtained by using titanium in the fertilizer either in the form of titanium sodium citrate or the insoluble sodium titanate. They found that the amount of phosphorus, silicon, and aluminum increased and decreased with the titanium content of the plants. The iron content, however, decreased with increasing applications of titanium. This led them to suggest that it might be possible that iron could be replaced by titanium in some plant metabolic processes.

In 1930 SIDERIS (**3**) reported that pineapple seedlings grown in a nutrient solution composed of

0.001 molar K_2HPO_4
0.002 molar KNO_3
0.001 molar $Ca(NO_3)_2$
0.001 molar $MgSO_4$

with five parts per million of titanium but no iron, formed chlorophyll just as well as when iron was added to the nutrient solution. SIDERIS used $TiCl_3$.

About two years ago we set up experiments in our laboratories with the object of testing the effect of titanous chloride ($TiCl_2$) on the formation of chlorophyll in *Zea mays*. We used the same concentration of nutrient solutions as was used by SIDERIS and added 5 and 10 p.p.m. of $TiCl_2$ instead of iron. Our controls consisted of nutrient solution with no titanium and no iron and nutrient solution with iron. This experiment has been repeated five times and 15 and 20 p.p.m. of titanium have been tried. All the results confirm the conclusion that titanous chloride will not substitute for iron in the formation of chlorophyll in *Zea mays*. Furthermore it can be readily seen from figure 1 that the growth of the tops and

[1] Contribution from the Kettering Foundation for the study of chlorophyll and photosynthesis.

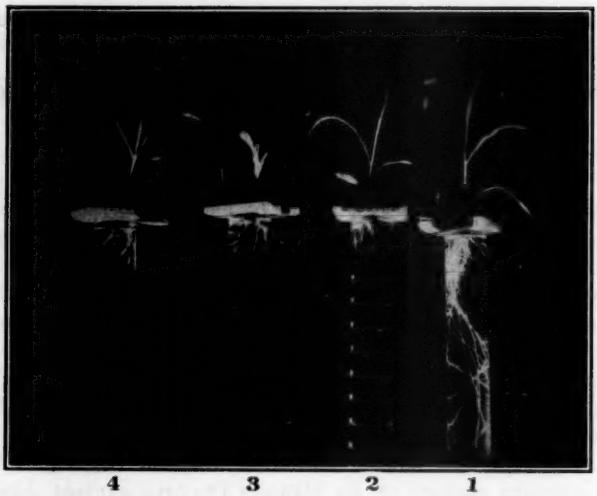

FIG. 1. Influence of titanous chloride and iron on the growth of *Zea mays*. 1, with trace of iron; 2, without iron; 3, with 5 p.p.m. $TiCl_3$ and no iron; 4, with 10 p.p.m. $TiCl_3$ and no iron.

the root systems is decidedly greater in the nutrient solution to which iron was added.

LEWIS (1) in a discussion of the genesis of the elements points out that nickel was most likely the parent substance for both iron and titanium. On this basis one might be inclined to favor the view that the common origin of iron and titanium might mean that there would be a good chance of substituting titanium for iron in chlorophyll formation in the plant. On the other hand it is most probable that iron acts as a catalyst in the formation of chlorophyll and owing to the fact that there is commonly clear cut specificity in such reactions it is unlikely that there could be a substitution of this type.

For the present this leaves us with no definite evidence that titanium has any value to the plant.—O. L. INMAN, GEORGE BARCLAY, and MALVERN HUBBARD, *Antioch College*.

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NOTES

St. Louis Meeting.—The twelfth annual meeting of the American Society of Plant Physiologists will be held in St. Louis from December 31, 1935 to January 2, 1936. The headquarters of the Society will be at the Statler Hotel. The program has been formulated by the program committee, and includes several sessions for the reading of papers. Joint meetings with the Horticulturists, with Section G, A. A. A. S., and with the Physiological Section of the Botanical Society of America have been scheduled. Appropriate periods for the transaction of necessary business have been provided. The annual dinner of the Society will be held on the evening of December 31. Tickets for the dinner should be obtained promptly on registration in order that accommodations for all may be provided. Concessions on the fares to St. Louis will be published by the A. A. A. S. in Science. Early hotel reservations are advisable because of the large attendance expected for this meeting.

Sixth International Botanical Congress.—The sixth International Botanical Congress which was held at Amsterdam early in September was attended by a large number of botanists from all parts of the world. The official representative of the Society at the Congress, Dr. WALTER F. LOEHWING of the University of Iowa, has consented to prepare an account of the meeting at Amsterdam for PLANT PHYSIOLOGY. If it is possible to do so, we hope to reproduce a photograph of the plant physiologists who were members of the congress.

Regional Section.—A petition for the organization of a new regional section of the American Society of Plant Physiologists will be presented to the executive committee and to the Society for action in the near future. The proposed section would include most of the territory west of the great plains, and Hawaii. Regional organization offers many advantages to the members residing within the region, and stimulates both the scientific and social life of the members. Additional information will be given following the St. Louis meeting.

Life Membership Committee.—The eleventh award of the CHARLES REID BARNES life membership will be made at the St. Louis meeting. The announcement will be made at the annual dinner, as has been the custom in past years. The committee whose duty it is to select the recipient of the award has been appointed by president MURNEEK, and is made up of the following members: Dr. JOHN W. SHIVE, chairman; Dr. E. C. MILLER, Dr. R. B. HARVEY, Dr. H. A. SPOEHR, and Dr. O. F. CURTIS.

Committee Chairmen.—In order to facilitate communications between the members of the Society and the committees which are maintained to carry on its work, the names of the chairmen of the various committees are presented here:

Executive Committee, Dr. A. E. MURNEEK, University of Misouri.
Editorial Committee, Dr. C. A. SHULL, University of Chicago.
Finance Committee, Dr. C. A. SHULL, University of Chicago.
Committee on Chemical Methods, Dr. W. E. TOTTINGHAM, University of Wisconsin.
Committee on Physical Methods, Dr. E. S. JOHNSTON, Smithsonian Institution, Washington, D. C.
Life Membership Committee, Dr. J. W. SHIVE, New Jersey Agricultural Experiment Station.
Program Committee, Dr. E. S. REYNOLDS, Missouri Botanic Garden.
Memorial Committee, Dr. F. M. ANDREWS, Indiana University.

Errata.—A few errors have been noted in the printing of volume 10 of PLANT PHYSIOLOGY. Those which have been found have been listed at the close of the table of contents. It is no doubt an incomplete list, as it has not been possible to devote time to reading the printed pages for this specific purpose. The authors of all papers are invited to examine their own work, and to report all errors found. It is regrettable that any errors slip by, but it is very difficult to avoid at least a few in each volume.

Praktikum der Zell- und Gewebephysiologie der Pflanze.—A new laboratory manual of plant physiology has been written by Dr. SIEGFRIED STRUGGER, Privatdozent in the University of Greifswald. This manual contains 94 experiments, which are arranged in eleven sections. These cover the main problems of general cellular physiology. The first section deals with the methods of preparation of living plant cells for experiment and observation. Then follow sections on plasmolytic phenomena; "intrability" (penetration, permeation, vital staining); permeability; vacuole contraction; plasmoptyse; secretion of pigments; nucleus; protoplasmic and cell sap viscosity; protoplasmic movements; and a section on regeneration, reproduction, and stimulation of cell division. The plants found useful in such experiments are listed in alphabetic order in an index, and a subject index completes the volume. The manual is illustrated with 103 cuts, and offers many good suggestions for successful experiments in the general physiology of plant cells. The publishers are Gebrüder Borntraeger, Berlin (W 35, Schöneberger Ufer 12a), who quote the price as RM 8.5 for cloth bound copies. If the usual foreign discount is allowed, this work is not unduly expensive.

Laboratory Plant Physiology.—A laboratory manual of plant physiology has been prepared by Dr. B. S. MEYER of Ohio State University and Dr. DONALD B. ANDERSON of the University of North Carolina. Part I is the manual, and part II is a supplementary section on laboratory methods. The manual presents 132 experiments, arranged in 18 sections. There are blank leaves for note-taking. The order of presentation is indicated by the sectional topics: Solutions; colloidal systems; plant cells; diffusion, osmosis, and imbition; permeability; the water relations of plant cells; the loss of water from plants; translocation of water; intake of water; the internal water relations of plants; the synthesis of basic carbohydrates; fat metabolism; the absorption and utilization of mineral salts; protein metabolism; digestion; translocation of solutes; assimilation and accumulation; and respiration. Lists of reference text books, manuals, and topical references for each section are included at the close of part I. The outline is attractive, and will be found quite useful. It is lithoprinted by the Edwards Brothers, Ann Arbor, Michigan, and may be purchased from them at a price of $1.75 per copy.

Problems in Soil Microbiology.—A brief monograph in the Rothamsted series on agricultural science has been written by Dr. D. WARD CUTLER and LETTICE M. CRUMP, both of the General Microbiology Department of the Rothamsted Experimental Station. It presents in substance the Aberystwyth Foundation lectures for the 1934–1935 session. There are seven chapters, 104 pages, 18 figures, and a map of protozoon distribution. The chapter headings are as follows: The suitability of the soil for microorganisms; the bacterial population under field conditions; the relation of bacteria to nitrite, carbon dioxide production by soil; the growth of protozoa in pure culture; the behavior of protozoa in soil; and the interactions between soil organisms. This is a very attractive short treatise on soil microbiology, and is an illuminating addition to the available soil monographs. The publishers are Longmans Green and Co., from whom it may be obtained at $3.20 per copy.

The Algae and their Life Relations.—The University of Minnesota Press has issued a volume on the fundamentals of phycology which considers the phylogeny of the algae, the distribution of marine forms in time and space, their classification (based on pigments and food reserves), control of algae in water supplies, and the utilization of algae as food for animals and man. The author of this work is Dr. JOSEPHINE E. TILDEN, professor of botany at the University of Minnesota, who has been a life-long student of the algae. The book is abundantly illustrated with 257 figures. A lengthy bibliography makes it extremely useful for those who desire to

consult the original sources of information. With the subject index it contains 550 pages, and is priced at $5.00 per copy. Dr. TILDEN has dedicated the book to her students, all of whom will appreciate this mark of her affection for them. The book will find many friends.

American Ferns.—An attractive and beautifully illustrated handbook for lovers and growers of ferns has been written by Dr. EDITH A. ROBERTS, Professor of Botany at Vassar College, and JULIA R. LAWRENCE. It presents in popular form just the information that fern growers need for proper management of their ferns. There is a brief introductory chapter on American ferns, and then chapters on how to know, grow, and use ferns in various types of decorative planting. Following these chapters, one finds a key for identification of ferns, a table indicating the time of spore collection and duration of greenness of the fronds, a time table for spore germination, thallus, formation, and appearance of the sporophyte, and a table showing the associational distribution of the species. A helpful glossary of terms is included. This little book will be popular with lovers of our fern flora who desire to know more about them, and to enjoy personal experiences in growing them. The publishers, Macmillan Co., quote it at $2.50 per copy.

Pansoma.—It is inevitable, perhaps, that biological philosophy and speculation is to undergo modification because of our changing ideas as to the ultimate nature of matter and energy. An attempt at the unification of our philosophical ideas has been made by Dr. A. C. LÉEMANN, of the University of Geneva, whose volume of speculation bears the challenging title: Pansoma et la géométrie de l'énergie. The pansome is defined as a three-dimensional energetic entity, indestructible and indivisible, bounded by pansomic surfaces, containing an absolute and constant energy, the only fundamental variability which it is capable of manifesting being that of changing its volume. Dr. LÉEMANN then applies the idea of the pansome to explanations of the physical structure of atoms, the phenomena of heat, ether, relativity, inertia, electricity, magnetism, and radiation.

He then passes on to apply the new philosophy to cosmology, causality, affinity and valence, crystallization, adsorption, the colloidal state, catalysis, vital phenomena, karyokinesis, organogenesis, genetics, mutations, and evolution. In the later sections he deals with nervous and muscular phenomena, paratonic reactions (tactisms and tropisms, etc.), and closes with an application of pansoma to psychology.

Here is an unlimited opportunity to enjoy a new philosophical thrill. The book is written in clear style, and it is broken into many brief sections which will facilitate reading and meditation. It comes in paper binding, and is issued by Libraire Georg & Co., Geneva, at a price of 15 Swiss francs.

Nations Can Live at Home.—Under this title Dr. O. W. WILLCOX in a small volume provides a solution for the problem of war. Incidentally the book emphasizes again his ideas as to how the agricultural lands of the earth should be utilized. There are eight chapters: The Malthusian bogey is extinct!; unfortunately, the Malthusian bogey is not yet extinct; when agronomic science is bankrupt; what the agrobiologist really has; delimiting the population problem; expanding the primary limit; peoples beyond the threshold (Great Britain, Italy, Germany, Japan); and the price of peace. An appendix presents crop yield as a phase of the mass action law. WILLCOX says wars are fought for foods and raw materials. Nations lack foods because of incompetent agriculture. To overcome this incompetence WILLCOX advocates the "divulgation of agrobiologic information." He advocates this especially for the "sore spots" of the earth where production is most ineffective and population pressure most grievous. By bringing in agrobiologic effectiveness, nations can live at home, and not need to war for their supplies.

It must not be forgotten, however, that nations that decide to be self-sufficient are bound to travel a hard, self-sacrificing road, which usually means a hard life for people generally, and a low standard of living. Nations *can* sometimes live at home, but it isn't a desirable life if it means that the nation is to depend entirely upon its own resources for everything. Even without population pressure we would still probably have wars. Incidentally, some of the assumptions made in WILLCOX's version of agrobiology do not stand close scrutiny. As a cure-all for the economic ills of mankind it would probably fail.

This volume is printed by the W. W. Norton Co., New York, and is one of a series of volumes edited by ALVIN JOHNSON. The price is $2.75 per copy.

GENERAL INDEX[1]

A

Abnormality, general aspects of leaf, 754
Absorption of sulphur dioxide by alfalfa and its relation to leaf injury, 291
  spectra of alpha and beta carotenes and lycopene, 375
Accumulation of boron by reciprocally grafted plants, 411
  of starch, 58
Acid hydrolysis, 77
  pantothenic as nutrilite for green plants, 559
Acids, fatty, before and after hydrolysis, 717
  organic, 458
Acalypha, 793
Address list, international, 409
Adsorption with decolorizing carbon, 139
Age of tissue, effect on osmotic pressure and water content, 675
Albinism, 182
Alcoholic extraction, 383
  storage, 144
ALDRICH, W. W., Influence of different quantities of moisture in a heavy soil on rate of growth of pears, 309
Alfalfa, absorption of sulphur dioxide and relation to leaf injury, 291
  salt concentration and reversibility of ice-formation related to hardiness, 403
Algae and their life relations, JOSEPHINE E. TILDEN, 825
Allium cepa, 209
Alpha carotene, 375
Aluminum, toxicity on seedlings, and action of certain ions in elimination of toxic effects, 1
American ferns, EDITH A. ROBERTS and JULIA R. LAWRENCE, 826
Amide nitrogen, 85, 396
Amino nitrogen, 82, 87, 394, 395
  nitrogen, determination in plant extracts, 135
Ammonia, 136
  organic acids as direct receptors, 458
Amylose, alpha and beta from starch, 715
Anabolic processes, soluble nitrogen as measure of available nitrogen, 355
Anaesthetics, 453
Analysis, chemical, of plant tissues, 383
Anatomical structure, 64
ANDERSON, D. B., Laboratory plant physiology, 825
Annual election, 581
Apparatus, 329, 330
  capillary siphon, 173
  constant level reservoir, 171
  cryoscopy, 663
  for control of nutrient solutions, 169
  for electrodialysis, 714
  for measuring leaf areas, 569
  for photosynthesis and respiration, 94
  for potential measurements, 31
  electrode chamber, 29
  reservoir connections, 174
  siphon shield, 176
Apple and pear fruits, comparison of changes, 594
  trees, fruiting relationships, 483
  varieties, physiology, 49
Area, leaf, 546
Areas, leaf, photoelectric apparatus for measuring, 569
Artemia salina, 429
Artichoke (Jerusalem), 414
Ash, 717, 746
Asparagine and nitrogen metabolism of plants, 447
Assimilation, effect of CO_2 concentration, 248
AUSTIN, J. P., Some effects of the photoperiod on development of *Impatiens balsamea*, 545
AUSTIN, STANLEY, Effects of exfloration on plant metabolism, 225
Avena sativa, temperature as a predetermining factor in development, 269
Award, STEPHEN HALES, 200
  life membership, 200
Axis, development of main and length of photoperiod, 549

[1] Papers published in PLANT PHYSIOLOGY are indexed in *The Agricultural Index*, The H. W. Wilson Co., New York.

B

BAKKE, A. L., Thermal conductivity of stored oats with different moisture content, 521
Balance, carbon dioxide, at high light intensities, 93
BARCLAY, G., Effect of titanous chloride on formation of chlorophyll in *Zea mays*, 821
Barley seedlings, development of chlorophyll and carotenoid pigments, 365
BARNES life membership award, 200
Bases, 733
Basic nitrogen, 86
BĚLEHRÁDEK, JAN, Temperature and living matter, 585
Beta carotene, 375
Bibliography, low temperature relations, R. B. HARVEY, 409
Biochemical laboratory methods, C. A. MORROW and W. M. SANDSTROM, 410
  phases of oleoresin production, 537
Biochemistry, annual review, 584
Bioelectric potential at flux equilibrium, kinetics of an intracellular system, 681
BLACKMAN'S law of limiting factors applied to photosynthesis, 245
BLAIR, G. Y., Accumulation of boron by reciprocally grafted plants, 411
Board, editorial, 200
Boron accumulation by reciprocally grafted plants, 411
Botanical congress, international, delegate, 409
  congress, international, 823
Buckwheat, 21
Budding and length of photoperiod, 547
Buffer capacity in relation to hardiness of plants, 149
BURR, G. O., Carbon dioxide balance at high light intensities, 93

C

Calcium deficiency effects on *Pisum sativum*, 811
Capacity, buffer, in relation to hardiness of plants, 149
Capillary siphons, 173
Carbohydrates, effects, 453
  determination, 387
  in *Elymus ambiguus* and *Muhlenbergia gracilis*, 727
Carbon dioxide balance at high light intensities, 93
Carotene and xanthophyll in pineapples, 187
  colorimetric determination in plant tissues, 325
Carotenes, alpha and beta, absorption spectra, 375
Carotenoid pigments, development in barley seedlings, 365
Cell E.M.F.'s, principle of summation, 209
  formation, stone, in pear fruits, 587
  oxidation and output of electric energy, 27
  physiology, 824
Cells, osmotic quantities of plants in given phases, 115
  plant, estimation of osmotic values of, 553
Chairmen, committee, 824
Change of tropic response, 196
Changes in nitrogen compounds in the wheat grain at different stages of development, 499
  seasonal, in composition of oak leaves, 739
Chemical analysis of plant tissues, 383
  methods committee, 409
Chemistry, stock-scion, and fruiting relationships in apple trees, 483
Cherries, observations on cracking, 763
CHICHESTER, D. F., Colorimetric determination of carotene in plant tissue, 325
Chlorate, sodium, in weed control, 699
Chloride, effect of titanous on formation of chlorophyll in *Zea mays*, 821
Chlorophyll content in pineapple plants, 159
Chlorophyll development in barley seedlings, 365
  effect of titanous chloride on formation, in *Zea mays*, 821
  formation and the beginning of photosynthesis, 401
Citrus limonia, 413

seedlings grown from x-rayed seed, growth modifications in, 179
Clearing methods, 387
CLEMENTS, F. E., Studies of the effect of artificial wind on growth and transpiration in *Helianthus annuus*, 613
Further studies of elongation and expansion in *Helianthus* phytometers, 637
COLBY, H. L., Stock-scion chemistry and fruiting relationships in apple trees, 483
Color with iodine, 716
Colorimetric determination of carotene in plant tissues, 325
Committee chairmen, 824
life membership, 823
chemical methods, 409
reports, 383, 387, 393
editorial, 408
finance, 409
program, 200
Compensation point, 107
Composition, chemical, of terminal shoots, 62
of oak leaves, seasonal changes, 739
Compounds, determination of nitrogen in simple, 393
Concentration, effect of CO_2 on assimilation, 248
Concentration of salt and hardiness of alfalfa, 403
Conductivity, electrical, 716
thermal, of oats, 521
Congress delegate, International Botanical, 409
International Botanical, 823
Containers and distribution of roots, 511
Content, chlorophyll, in pineapple plants, 159
moisture, and thermal conductivity of oats, 521
sucrose, of prunes, 575
sulphur, of plants, 529
water, of prairie plants, 661
Continuous flow of nutrient solutions, mechanism for, 169
Controlled continuous flow of nutrient solutions, mechanism for, 169
Control of weeds and sodium chlorate, 699
COOPER, W. C., Hormones in relation to root formation on stem cuttings, 789
Corn, 6, 401
polysaccharides in sweet, 713
tissues, solubility of potassium in, 565
Corresponding members, 199
Cost of reprints, 201
Cracking of cherries, 763
CRAFTS, A. S., Physiological problems connected with the use of sodium chlorate in weed control, 699
CRUMP, L. M., Problems in soil microbiology, 825
Cryoscopy, apparatus, 663
CUTLER, D. S., Problems in soil microbiology, 825
Cuttings, hormones in relation to root formation on stem, 789

D

DAVIDSON, O. W., Determination of nitrogenous fractions in vegetative tissue of peach, 73
DAY, DOROTHY, Some chemical aspects of calcium deficiency effects on *Pisum sativum*, 811
Deemulsifiers, 385
Deficiency, calcium, effects on *Pisum sativum*, 811
Defoamers, 385
Deleading, 387
Delegate, International Botanical Congress, 409
Determination, colorimetric of carotene in plant tissue, 325
of amino nitrogen in plant extracts, 135
of carbohydrates, 387
of nitrogen in relatively simple compounds, 393
of nitrogenous fractions in vegetative tissue of peach, 73
of starch in plant tissue, 579
Determinations, sugar, 388
Determining nitrogen in plants, 355
Development of *Avena sativa*, 269
of cholorphyll and carotenoid pigments in barley seedlings, 365
of *Impatiens balsamea*, 545

of main axis and length of photoperiod, 549
nitrogen compounds in wheat grain at different stages, 499
DEVRIES, HUGO, obit., 584
DEXTER, S. T., Growth, organic nitrogen fractions, and buffer capacity in relation to hardiness of plants, 149
Salt concentration and reversibility of ice-formation as related to hardiness of alfalfa, 403
Dextrine, 389
Dextrose-levulose ratio of prunes, 575
Dioxide, carbon, balance at high light intensities, 93
Distillation with solid calcium oxide, 139
Distribution of roots in porous and nonporous plant containers, 511
Dormancy of lettuce seeds and light, 193
Drying, heat, 384
Duplication, 184

E

EATON, F. M., Accumulation of boron by reciprocally grafted plants, 411
Edestin, 503
Editorial board, 200
committee, 408
Effects of photoperiod on development of *Impatiens balsamea*, 545
EISENMENGER, W. S., Toxicity of aluminum on seedlings and action of certain ions in elimination of toxic effects, 1
Election, annual, 581
Electric energy, linkage between output by polar tissues and cell oxidation, 27
Electrodialysis, apparatus for, 714
Electromotive force, principle of summation of cell, 209
Elongation in *Helianthus* phytometers, 637
Elymus ambiguus, seasonal march of carbohydrates, 727
EMMERT, E. M., Method for quickly determining nitrogen in plants, and soluble nitrogen as a measure of nitrogen available for anabolic processes, 355
Endowments, 201
Energy, electric, linkage between output by polar tissues and cell oxidation, 27
ENSIGN, M. R., Factors influencing growth and yield of potatoes in Florida, 465
Enzymatic hydrolysis, 78
Equilibrium, flux, kinetics of an intracellular system for respiration and bioelectric potential, 681
water, in plant tissues, 781
ERNEST, ELIZABETH C. M., Factors rendering the plasmolytic method inapplicable in the estimation of osmotic values of plant cells, 553
Errata, 824
Estimation of osmotic values of plant cells, 553
Excretion, A. FREY-WYSSLING, 202
Exfloration, effects on plant metabolism, 225
Exosmosis method, 149
Expansion in *Helianthus* phytometers, 637
Extracting, method, 74
Extraction, 384
alcoholic, 135, 383
water, 135
of pigments, 326
Extractives, water and 80 per cent. alcohol as, 145
Extract, nitrogen-free, 746
Extracts, determination of amino nitrogen in plants, 135

F

Factor, temperature as a predetermining in development of *Avena sativa*, 269
Factors, limiting, and photosynthesis, 245
Fasciation, 183
Ferns, American, EDITH A. ROBERTS and JULIA R. LAWRENCE, 826
Fertilization, nitrogen, and chlorophyll content in pineapple plants, 159
Fiber, crude, 746
Fig, 793
Finance committee, 409
FINCH, A. H., Physiology of apple varieties, 49
FISHER, R. A., Statistical methods, 202
Flax, 246
Flocculation by salts, 716
Florida, growth and yield of potatoes, 465

Flow of nutrient solutions, mechanism for controlled continuous, 169
streamline, and movement of solutes in transpiration stream, 773
Flowering and length of photoperiod, 547
premature, 181
Flux equilibrium, kinetics of an intracellular system for respiration and bioelectric potential, 681
Formation of chlorophyll and beginning of photosynthesis, 401
of chlorophyll in *Zea mays*, 821
root, on stem cuttings, hormones in relation, 789
stone cell, in pear fruits, 587
Fractionation of nitrogen, 393
Fractions, nitrogenous, in vegetative tissue of peach, 73
organic nitrogen in relation to hardiness of plants, 149
FRAZER, JOHN, Sulphur content of plants, 529
FREAR, D. E. H., Photoelectric apparatus for measuring leaf areas, 569
Freezable water equilibrium in plant tissues, 781
Freezing, 383
FREY-WYSSLING, A., Die Stoffausscheidung der höheren Pflanzen, 202
Frog nerve, 220
Fructose, separation, 389
Fruiting relationships in apple trees, 483
Fruits, stone cell formation in pear, 587
Fuel oil, effects on plants, 817
FULLER, G. D., Some effects of fuel oil on plants, 817

G

GERRY, ELOISE, Biochemical phases of oleoresin production, 537
Gliadin, 499
Glucose and fructose, separation, 389
Glutenin, 499
GOEBEL, KARL RITTER VON, biographical sketch, 203
portraits, opposite pp. 203, 205
Grafted plants, 411
Grain, wheat, nitrogen compounds, 499
Grapefruit, 179
Grazing use, seasonal march of carbohydrates in *Elymus ambiguus* and *Muhlenbergia gracilis*, and their reaction under moderate, 727
GREATHOUSE, G. A., Unfreezable and freezable water equilibrium in plant tisues as influenced by sub-zero temperatures, 781
Green plants, pantothenic acid as a nutrilite, 559
Grimmia montana, photosynthesis, 803
Growth, character of terminal shoot, 54
in *Helianthus annuus*, effect of artificial wind, 613
in relation to hardiness of plants, 149
modifications in citrus seedlings grown from x-rayed seed, 179
of pears, influence of different quantities of moisture in a heavy soil on rate, 309
of plants, 729
of oak leaves, 739
of potatoes in Florida, 465

H

HALES, STEPHEN, award, 200
HALL, J. A., Biochemical phases of oleoresin production, 537
HARDER'S concept of relative minimum and photosynthesis, 245
Hardiness of alfalfa, salt concentration, and reversibility of related ice formation, 403
of plants, growth, organic nitrogen fractions and relation of buffer capacity, 149
Hardness, of pear and apple, 602
HARVEY, R. B., Low temperature relations bibliography, 409
HASKINS, C. P., Growth modifications in citrus seedlings grown from x-rayed seed, 179
HASKINS, H. D., Distribution of roots in porous and non-porous plant containers, 511
Heat drying, 384
Helianthus annuus, 413
tuberosus, 413
annuus, effect of artificial wind on growth and transpiration, 613
annuus, effect of solar radiation on transpiration, 341

phytometers, elongation and expansion, 637
Herbage, 731
HILL, G. R., JR., Absorption of sulphur dioxide by alfalfa and its relation to leaf injury, 291
HIMMEL, W. J., A case of change of tropic response, 196
Hormones in relation to root formation on stem cuttings, 789
HUBBARD, MALVERN, Effect of titanous chloride on the formation of chlorophyll in *Zea mays*, 821
Humidity as a factor influencing osmotic pressure, 671
Humin nitrogen, 86
HUTCHINGS, L. R., Streamline flow and movement of solutes in transpiration stream, 773
Hydrolysis of sucrose, 388

I

Ice-formation and hardiness of alfalfa, 403
Impatiens balsamea, effects of photoperiod on development, 545
Incipient plasmolysis, 554
Injury to leaves and absorption of sulphur dioxide in alfalfa, 291
INMAN, O. L., Formation of chlorophyll and beginning of photosynthesis, 401
Effect of titanous chloride on formation of chlorophyll in *Zea mays*, 821
Intensities, light, carbon dioxide balance at high, 93
of light, 254
International address list, 409
Botanical Congress, 823
Botanical Congress delegate, 409
Intracellular system for respiration and bioelectric potential at flux equilibrium, 681
Iodine, color, 716
Ions, action of in elimination of toxic effects of aluminum on seedlings, 1
Isolation of starch in plant tissue, 579

J

JONES, L. H., Distribution of roots in porous and non-porous plant containers, 511

K

KERTESZ, Z. I., Observations on the cracking of cherries, 763
Kinetics of an intracellular system for respiration and bioelectric potential at flux equilibrium, 681

L

Laboratory manual of cell and tissue physiology of plants, SIEGFRIED STRUGGER, 824
methods, biochemical, 410
plant physiology, B. S. MEYER and D. B. ANDERSON, 825
Lakes of high magnesium sulphate content and salt tolerance of *Ruppia maritima*, 425
LAL, K. N., Limitations of BLACKMAN'S law of limiting factors and HARDER'S concept of relative minimum as applied to photosynthesis, 245
Lantana, 793
LAWRENCE, JULIA R., American ferns, 826
LEADBEATER, MARGARET R., Some effects of fuel oil on plants, 817
Leaf areas, photoelectric apparatus for measuring, 569
formation, peloric, 185
injury and absorption of sulphur dioxide in alfalfa, 291
structure, modification by x-rays, 753
Leaves, oak, growth and seasonal changes in composition, 739
LÉEMANN, A. C., Pansoma, 826
Lemon, 179, 415
experiments, 789
Lettuce seeds, light as a factor influencing the dormancy, 193
Leucosin, 503
Levulose-dextrose ratio of prunes, 575
LEWIS, M. R., Influence of different quantities of moisture in a heavy soil on rate of growth of pears, 309
Life membership, BARNES, 200
membership committee, 823
relations of algae, JOSEPHINE E. TILDEN, 825
Light as a factor influencing the dormancy of lettuce seeds, 193

intensities, carbon dioxide balance, 93
intensity, 254
Lignocellulose, 587
Lime, 179
Limiting factors and photosynthesis, 245
Linkage between output of electric energy in polar tissues and cell oxidation, 27
LINK, K. P., Isolation and determination of starch in plant tissue, 579
List, international address, 409
Living matter and temperature, JAN BĚLEHRÁDEK, 585
LLOYD, F. E., biographical sketch of KARL RITTER VON GOEBEL, 203
Localization of injuries induced by x-rays, 760
LONG, FRANCES L., Further studies of elongation and expansion in *Helianthus* phytometers, 637
Longleaf pine, 537
Low temperature relations bibliography, R. B. HARVEY, 409
LUND, E. J., Linkage between output of electric energy by polar tissues and cell oxidation, 27
Lycopene, absorption spectra of, 375

M

MACKINNEY, G., Development of the chlorophyll and carotenoid pigments in barley seedlings, 365
Absorption spectra of alpha and beta carotenes and lycopene, 375
MCCARTY, E. C., Seasonal march of carbohydrates in *Elymus ambiguus* and *Muhlenbergia gracilis*, and their reaction under moderate grazing use, 727
MCKAY, ELIZABETH, Salt tolerance of *Ruppia maritima* in lakes of high magnesium sulphate content, 425
Photosynthesis in *Grimmia montana*, 803
MCLEAN, R. C., Streamline flow and movement of solutes in transpiration stream, 773
MAGISTAD, O. C., Relationship between nitrogen fertilization and chlorophyll content in pineapple plants, 159
Carotene and xanthophyll in pineapples, 187
Magnesium sulphate content, salt tolerance of *Ruppia maritima* in lakes of high, 425
March, seasonal, of carbohydrates, 727
MARSH, G., Kinetics of an intracellular system for respiration and bioelectric potential at flux equilibrium, 681
MARTIN, E. V., Effect of solar radiation on transpiration of *Helianthus annuus*, 341
Studies of the effects of artificial wind on growth and transpiration in *Helianthus annuus*, 613
Matter, living, and temperature, JAN BĚLEHRÁDEK, 585
Maturation period, 748
Mechanism for controlled continuous flow of nutrient solutions, 169
Meeting, St. Louis, 408, 581, 823,
Minneapolis, 581
Pittsburgh, 199
Summer, 199
Meetings, summer, 407
MEHRLICH, F. P., A mechanism for controlled continuous flow of nutrient solutions, 169
Melting point of osazones, 717
Membership committee, life, 823
life, 200
Members, corresponding, 199
Mesophyll structure, 656
Metabolism, effects of exfloration on plant, 225
nitrogen, of plants, rôle of asparagine, 447
Methods, biochemical laboratory, C. A. MORROW and W. M. SANDSTROM, 410
committee, chemical, 409
plasmolytic, and estimation of osmotic values of plant cells, 553
statistical, 202
MEYER, B. S., Laboratory plant physiology, 825
Microbiology of soil, D. S. CUTLER and L. M. CRUMP, 825
MILLER, E. S., Carbon dioxide balance at high light intensities, 93
Absorption spectra of alpha and beta carotenes and lycopene, 375
Minimum, relative, and photosynthesis, 245
Minneapolis meeting, 581

Minnesota section, 408
Modification, structural, of sunflower, 652
Modifications of growth in citrus seedlings grown from x-rayed seed, 179
Moisture content and thermal conductivity of oats, 521
in a heavy soil, and rate of growth of pears, 309
soil, as a factor influencing osmotic pressure, 671
MOORE, C. N., Growth modifications in citrus seedlings grown from x-rayed seed, 179
MORRIS, V. H., Solubility of potassium in corn tissues, 565
MORROW, C. A., Biochemical laboratory methods, 410
Movement of solutes in the transpiration stream, 773
Muhlenbergia gracilis, carbohydrates, 727
MURNEEK, A. E., Physiological rôle of asparagine and related substances in nitrogen metabolism of plants, 447

N

Nations can live at home, O. W. WILLCOX, 827
NEBEL, B. R., Observations on the cracking of cherries, 763
Nerve, frog, 220
New England section, 582
NICHOLS, P. F., Notes on the sucrose content and dextrose-levulose ratio of California dried prunes, 575
NIEMANN, CARL, Isolation and determination of starch in plant tissue, 579
Nitrate nitrogen, 86, 396
Nitrates, 393
Nitrogen, amide, 85
amino, 87
ammonium, 82
basic, 86
cyanogenetic, 76
compounds in wheat, 499
content of tree tissues, 486
determination of amino in plant extracts, 135
determination in plants, 355
determination in relatively simple compounds, 393
fertilization and chlorophyll content in pineapple plants, 159
fractions, organic, in relation to hardiness of plants, 149
humin, 86
nitrate, 86
metabolism of plants and rôle of asparagine, 447
nutrient, response to high and low, 67
total soluble, 84
Nitrogenous fractions in vegetative tissue of the peach, 73
NOGUCHI, Y., Modification of leaf structure by x-rays, 753
Non-porous plant containers, distribution of roots, 511
Nutrient solutions, mechanism for controlled continuous flow, 169
Nutrilite for green plants, pantothenic acid as, 559

O

Oak leaves, growth and seasonal changes in composition, 739
Oaks, stomata and transpiration, 795
Oats, 269
thermal conductivity of, 521
Oil, fuel, effects on plants, 817
Oleoresin production, 537
Orange, 179
Organic nitrogen fractions in relation to hardiness of plants, 149
Osazones, melting point, 717
Osmotic pressure and water content of prairie plants, 661
quantities of plant cells in given phases, 115
values of plant cells, estimation, 553
Oxidation, 452
cell, and output of electric energy, 27

P

Pansoma, A. C. LÉEMANN, 826
Pantothenic acid as a nutrilite for green plants, 559
Paradise apple stock, 483
PARKER, M. W., Physical and chemical properties of the soluble polysaccharides in sweet corn, 713

Pea, Canada field, 811
Peach, determination of the nitrogenous fractions in vegetative tissue, 73
Peaches, 819
Pear fruits, stone cell formation, 587
Pears, influence of different quantities of moisture in heavy soil on rate of growth, 309
Peloric leaf formation, 185
Permanence of carotene and xanthophyll in canned pineapple, 190
Phases, biochemical, of oleoresin production, 537
osmotic quantities of plant cells in given, 115
Phenols, derivatives of, 137
Phosphorus, 717
phosphate, effect of soluble nitrogen, 362
Photoelectric apparatus for measuring leaf areas, 569
Photoperiod, effects on development of *Impatiens balsamea*, 545
Photosynthesis, 93
beginning of and the formation of chlorophyll, 401
BLACKMAN'S law of limiting factors and HARDER'S concept of relative minimum applied, 245
in *Grimmia montana*, 803
Physiology, of apple varieties, 49
plant, laboratory, 825
Phytometers, elongation and expansion in *Helianthus*, 637
Pigments, carotenoid, development in barley seedlings, 365
Pineapple plants, relationship between nitrogen fertilization and chlorophyll content, 159
xanthophyll, 187
Pine, longleaf, 537
slash, 537
Pisum sativum, calcium deficiency effects, 811
Pittsburgh meeting, 199
Plant cells, estimation of osmotic values of, 553
cells, osmotic quantities of in given phases, 115
containers, distribution of roots, 511
extracts, determination of amino nitrogen, 135
physiology, laboratory, 825
metabolism, effects of exfloration, 225
tissue, chemical analysis, 383
tissue, colorimetric determination of carotene, 325
tissue, isolation and determination of starch, 579
Plants, determining nitrogen, 355
effect of fuel oil, 817
growth, organic nitrogen fractions, and buffer capacity in relation to hardiness, 149
laboratory manual of cell and tissue physiology, SIEGFRIED STRUGGER, 824
pantothenic acid as a nutrilite, 559
prairie, osmotic pressure and water content, 661
relationship between nitrogen fertilization and chlorophyll content in pineapple, 159
rôle of asparagine and related substances in nitrogen metabolism, 447
sulphur content, 529
Plasmolytic method and estimation of osmotic values of plant cells, 553
PLITT, THORA M., Temperature as a predetermining factor in development of *Avena sativa*, 269
Polarity, electrical, 686
Polar tissues and cell oxidation, linkage between output of electric energy by, 27
Polysaccharides in sweet corn, 713
Porous plant containers, distribution of roots in, 511
Portraits, 201
GOEBEL, frontispiece opp. p. 203
GOEBEL, opp. p. 205
Potassium in corn tissues, 565
in external solution and pigment formation, 368
Potatoes, growth and yield in Florida, 465
Potential, bioelectric, at flux equilibrium, kinetics of an intracellular system, 681
Prairie plants, osmotic pressure and water content, 661

Precipitation with neutral lead acetate solution, 139
Predetermining factor, temperature as, in development of *Avena sativa*, 269
Premature flowering of citrus, 181
Preparation of tissue for extraction, 74
Preservation, 383
Pressure, osmotic, of prairie plants, 661
Principle of summation of cell E.M.F's, 209
Processes, soluble nitrogen as a measure of nitrogen available for anabolic, 355
Production of oleoresin, 537
Program committee, 200
Proof of the principle of summation of E.M.F's, 209
Prunes, sucrose content and dextrose-levulose ratio, 575

Q

Quantities, osmotic, of plant cells in given phases, 115
Quercus, 739, 795

R

Radiation, solar, and transpiration of *Helianthus annuus*, 341
Ratio, dextrose-levulose, of prunes, 575
Ratios, top-root, in containers, 511
Receptors of ammonia, organic acids as direct, 458
Reciprocal grafts, 411
Reduction of FEHLING'S solution before and after hydrolysis, 717
Regional section, 823
Relations, low temperature bibliography, R. B. HARVEY, 409
  water, reprints wanted, 201
Report, chemical methods committee, 409
  committee on chemical methods, 383
Reprints, cost, 201
Respiration, 93
  at flux equilibrium, kinetics of an intracellular system, 681
Response, change of tropic, 196
  to high and low nitrogen nutrient, 67
Reversibility of ice-formation and hardiness of alfalfa, 403
Review of biochemistry, annual, 584
Rhizomes, 733
Ricciocarpus natans, 559
ROBERTS, EDITH A., American ferns, 826
ROBERTS, R. H., Isolation and determination of starch in plant tissue, 579
ROHRMAN, EWALD, Pantothenic acid as a nutrilite for green plants, 559
Root formation on stem cuttings, hormones in relation, 789
Roots, 733
  and tops, comparative values, 675
  distribution in containers, 511
ROSENE, H. F., Linkage between output of electric energy by polar tissues and cell oxidation, 27
  Proof of the principle of summation of cell E.M.F.'s, 209
Rotation, optical, 716
Ruppia maritima, salt tolerance in lakes of high magnesium sulphate content, 425
RUSSELL, W. C., Colorimetric determination of carotene in plant tissue, 325

S

Salt concentration and hardiness of alfalfa, 403
  tolerance of *Ruppia maritima* in lakes of high magnesium sulphate content, 425
SAMISCH, R., Growth and seasonal changes in composition of oak leaves, 739
Sampling, 51, 383
  methods, 161
SAMPSON, A. W., Growth and seasonal changes in composition of oak leaves, 739
SANDSTROM, W. M. Biochemical laboratory methods, 410
SAYRE, J. D., Solubility of potassium in corn tissues, 565
Scion (stock) chemistry and fruiting relationships in apple trees, 483
Secretion, A. FREY-WYSSLING, 202
Section, Minnesota, 408
  New England, 582
  Purdue, 583
  regional, 823

Seed, growth modifications in citrus seedlings grown from x-rayed, 179
light as a factor influencing the dormancy of lettuce, 193
Seedlings, barley, development of the chlorophyll and carotenoid pigments, 365
citrus, grown from x-rayed seed, 179
toxicity of aluminum, 1
Senescence period, 749
Separation of glucose and fructose, 389
of pigments, 328
Severinia buxifolia, 413
SHIVE, J. W., Determinations of nitrogenous fractions in vegetative tissue of peach, 73
Shoots, chemical composition of terminal, 62
SHUCK, A. L., Light as a factor influencing dormancy of lettuce seeds, 193
SINGH, B. N., Limitations of BLACKMAN'S law of limiting factors and HARDER'S concept of relative minimum as applied to photosynthesis, 245
Siphons, capillary, 173
Slash pine, 537
SMITH, W. W., The course of stone cell formation in pear fruits, 587
Sodium chlorate in weed control, 699
Soil microbiology, 825
moisture as a factor influencing osmotic pressure, 671
Solar radiation and transpiration of *Helianthus annuus*, 341
Solubility of potassium in corn tissues, 565
Solutes, movement in the transpiration stream, 773
Solutions, nutrient, mechanism for controlled continuous flow, 169
Soy bean, 14, 225
Spectra, absorption, of alpha and beta carotenes and lycopene, 375
Stages of development, changes in nitrogen compounds in wheat grain, 499
Starch, 390
accumulation, 58
content of tree tissues, 488
disappearance and accumulation, 51
isolation and determination in plant tissue, 579
Statistical methods, R. A. FISHER, 202
Stem cuttings, hormones in relation to root formation, 789
STEPHEN HALES award, 200
STILES, H., Thermal conductivity of stored oats with different moisture content, 521
Stimulation and nutrilites, 562
St. Louis meeting, 408, 581, 823
Stock-scion chemistry and fruiting relationships of apple trees, 483
STODDART, L. A., Osmotic pressure and water content of prairie plants, 661
Stomata, 622, 653
of oaks, 795
Stone cell formation in pear fruits, 587
Storage studies, 604
Streamline flow and movement of solutes in transpiration stream, 773
Stream, streamline flow and movement of solutes in transpiration, 773
Structural modification of sunflower, 652
Structure, anatomical, 64
leaf, modification by x-rays, 753
mesophyll, 656
STRUGGER, SIEGFRIED, Praktikum der Zell- und Gewebephysiologie der Pflanze, 824
STUART, N. W., Determination of amino nitrogen in plant extracts, 135
Sucrose, content of prunes, 575
hydrolysis, 388
Sugar cane, 246
determinations, 388
Sulphate, magnesium, content, salt tolerance of *Ruppia maritima* in lakes of high, 425
Sulphur content of plants, 529
dioxide, 291
Summation of cell E.M.F's, 209
Summer meetings, 199, 407
Sunflower, 341, 613, 637, 753
structural modification, 652
Sunflowers, 414
Sweet corn, polysaccharides, physical and chemical properties, 713
Synthesis of asparagine from NH_3 and organic acids, 456
System, intracellular, for respiration and bioelectric potential at flux equilibrium, kinetics, 681

T

TAM, R. K., Relationship between nitrogen fertilizer and chlorophyll content in pineapple plants, 159
Tangerine, 179
TAYLOR, M. W., Colorimetric determination of carotene in plant tissue, 325
TELLER, G. L., Changes in nitrogen compounds in wheat grain at different stages of development, 499
Temperature, 151
  and assimilation, 260
  and CO_2 exchange, 96
  and living matter, JAN BĚLEHRÁDEK, 585
  as a predetermining factor in development of *Avena sativa*, 269
  effect on yield and grade of potato tubers, 475
  relations bibliography, low, R. B. HARVEY, 409
Temperatures, unfreezable and freezable water equilibrium in plant tissues as influenced by sub-zero, 781
Thermal conductivity of stored oats with different moisture content, 521
THOMAS, M. D., Absorption of sulphur dioxide by alfalfa and its relation to leaf injury, 291
TILDEN, JOSEPHINE E., The algae and their life relations, 825
Tissue, chemical analysis of plant, 383
  of peach, nitrogenous fractions in vegetative, 73
  physiology, 824
  plant, and starch, 579
  plant, colorimetric determination of carotene, 325
Tissues, corn, solubility of potassium in, 565
  polar, and output of electric energy, 27
Titanous chloride, effect on formation of chlorophyll in *Zea mays*, 821
Tolerance, salt, of *Ruppia maritima* in lakes of high magnesium sulphate content, 425
Tomatoes, 818
Tops and roots, comparative values, 675
Toxicity of aluminum on seedlings and action of certain ions in the elimination of the toxic effects, 1
Tradescantia, 793
Transpiration in *Helianthus annuus* and solar radiation, 341
  in *Helianthus annuus*, effect of artificial wind, 613
  of oaks, 795
  stream, streamline flow and movement of solutes, 773
Trees, apple, fruiting relationships, 483
Tropic response, change, 196
Twisting, 183

U

Unfreezable water equilibrium in plant tissues, 781
Urea formation and other means of removing NH_3, 459
URSPRUNG, ALFRED, Osmotic quantities of plant cells in given phases, 115

V

Values, estimation of osmotic, of plant cells, 553
Vegetative tissue of the peach, nitrogenous fractions in, 73
Vigor, relation of soluble nitrogen and phosphate phosphorus to plant, 362
Viscosity, 716

W

Water content of prairie plants, 661
  equilibrium in plant tissues, 781
  relations, reprints wanted, 201
Weed control and sodium chlorate, 699
WENT, F. A. F. C., obit, 584
Wheat, 150, 246
  grain, nitrogen compounds, 499
WILLCOX, O. W., Nations can live at home, 827
WILLIAMS, R. J., Pantothenic acid as a nutrilite for green plants, 559
Wind, effect of artificial on growth and transpiration in *Helianthus annuus*, 613

WORK, R. A., Influence of different quantities of moisture in heavy soil on rate of growth of pears, 309

X

Xanthophyll in pineapples, 187
X-rayed seed, growth modifications in citrus seedlings, grown from, 179
X-rays, modification of leaf structure, 753
Xylem formation, 58
formation and starch disappearance and accumulation, 51

Y

Yield of plants, relation of soluble nitrogen and phosphate phosphorus, 362
of potatoes in Florida, 465
YOCUM, L. E., The stomata and transpiration of oaks, 795

Z

Zea mays, formation of chlorophyll, 821
ZSCHEILE, F. P., Jr., Absorption spectra of alpha and beta carotenes and lycopene, 375